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Use of water hyacinth-based compost to improve the growth and biochemical properties of lettuce (*Lactuca sativa var. crispa* L.)

Jutarut Iewkittayakorn[1, *], Aunkamol Kumngen[2], Usmana Meehae[2], Pattarawadee Wongsuwan[2], Siwapong Leunram[2], and Juntima Chungsiriporn[3]

[1]Division of Biological Science, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand
[2]BIOAXEL Co., Ltd. 6/9 Moo 3 Bophut Chaweng Beach Road, Koh Sa-mui, Surat Thani 84320, Thailand
[3]Division of Chemical Engineering, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand
[*]Corresponding author: jutarut.p@psu.ac.th

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Abstract

Water hyacinth (*Eichhornia crassipes*) is an invasive aquatic weed that spreads rapidly and poses serious ecological threats to freshwater ecosystems. Converting this biomass into compost offers a sustainable solution for weed management and soil fertility improvement. This study evaluated the effects of water hyacinth-based (WH-based) compost on the growth and biochemical properties of lettuce (*Lactuca sativa var. crispa* L.). Lettuce plants were cultivated for 45 days in a planting medium composed of 70% (v/v) topsoil and 30% (v/v) coconut coir, amended with 0%, 10%, 30%, 50%, 70%, and 90% (v/v) of WH-based compost. Two commercial soil media without compost served as controls. Growth and biochemical parameters were analyzed using one-way ANOVA, and means were compared with Tukey's HSD test at $p < 0.05$. The 10% compost treatment produced the highest growth parameters, with a plant length of 27.33 ± 1.53 cm, a root length of 18.33 ± 1.53 cm, a leaf number of 26.83 ± 0.76, a bush width of 28.00 ± 1.00 mm, a plant thickness of 19.23 ± 0.73 mm, a leaf weight of 98.29 ± 1.00 g, and a dry weight of 4.93 ± 0.03 g. Additionally, 10% compost significantly increased protein, carbohydrate, and reducing sugar contents. Overall, WH-based compost at appropriate ratios can effectively enhance lettuce growth and biochemical quality while providing an eco-friendly approach to the management water hyacinth biomass.

Keywords: Biochemical analyzes, Compost, Lettuce, Water hyacinth, Waste-recycling

1. Introduction

Water hyacinth (WH) (*Eichhornia crassipes*) is an invasive aquatic weed. In Thailand, WH poses a serious threat to ecosystems, agriculture, irrigation systems and public health due to its rapid spread and resistance to environmental conditions [1]. The Thai government has addressed this problem by initiating projects, such as the "Beautiful City, Clean Water" project, to control and reduce WH in public water sources [2]. Despite the use of manual, mechanical and herbicidal control methods, WH continues to spread. However, the utilization of WH biomass as compost is a sustainable and environmentally friendly alternative that could turn WH into a valuable resource. Studies have been conducted on the potential of WH compost as a soil amendment for crop production. Its high organic matter content and balanced nutrient profile make it an attractive alternative to chemical fertilizers, especially for sustainable agricultural practices. Olayiwola et al. [3] reported that WH compost can improve soil fertility and promote the growth of maize, leading to an increase in biomass and yield. Sangeetha and Thevanathan [4] also reported that treatment of okra (*Abelmoschus esculentus*) with WH compost significantly improved vegetative growth parameters, including plant height and leaf number, compared to untreated control plants. In addition, Jain and Kalamdhad [5] found that WH compost mixed with cow dung improved seed germination, root development and chlorophyll content of spinach. These results indicated that composted WH not only provides important macro- and micronutrients, but also improves soil texture and water retention capacity, which are important factors for plant growth. Beyond its agronomic benefits, the use of WH compost offers several environmental and economic advantages. Environmentally, composting WH helps reduce aquatic weed waste, minimize landfill burden, and lower greenhouse gas emissions associated with uncontrolled decomposition. It also contributes to improving soil health through increased organic matter, enhanced microbial activity, and improved nutrient cycling. Economically, WH compost production and use can reduce farmers' dependence on costly chemical fertilizers, thus lowering production costs and promoting a circular economy by converting locally abundant biomass into a marketable fertilizer product. Such utilization not only supports environmentally responsible waste management but also strengthens community-based agricultural sustainability.

Oakleaf lettuce (*Lactuca sativa var. crispa* L.) is a popular leafy vegetable that is usually eaten fresh, has economic potential and high nutritional value as it contains vitamins A and C, minerals (especially calcium), antioxidants (quercetin, caffeic acid) and other active compounds [6]. Lettuce thrives at low temperatures and a slightly acidic to neutral pH (6–6.5) [7, 8]. Tharungsri et al [9] investigated the effect of fermented WH and residual waste on germination and growth of green oakleaf lettuce using seven treatments including soil (control), peat moss and five different formulas of fermented WH mixtures. The results showed that the appropriate ratio of fermented WH, bagasse, criket manure and vinasse for use as planting material was 30:25:25:20, which resulted in the highest growth of green oakleaf lettuce. Tharangsri et al [10] studied the effect of a compost mixture containing elephant dung, hay dung fertilizer, WH, vinasse and bat guano on the growth of green oakleaf lettuce. The result was that a mixture of 40% elephant dung, 30% cricket dung, 20% WH and 10% vinasse produced the highest growth and yield values, which were comparable to those of chemical fertilizers.

Despite these promising findings, few studies have specifically examined the biochemical effects of WH compost on the nutritional quality of lettuce. Therefore, the aim of this study is to investigate both the growth performance and biochemical composition of lettuce cultivated with WH compost added to the planting material at different ratios to determine the optimal ratio for lettuce cultivation. The results are expected to provide practical insights into the use of WH compost as a sustainable fertilizer alternative for lettuce cultivation.

2. Materials and methods

2.1 Composting of water hyacinth

Fresh WH was collected from irrigation canals and swamps unaffected by sewage in Hat Yai, Songkhla, Thailand. The WH was cut with a knife into small pieces approximately 5–10 cm in length, and subsequently fed into an organic waste composting machine (Bio Axel Co., Ltd., Thailand). Inside the machine, the WH was mixed with a specialized composting mixture consisting of several Bacillus species in rice bran. The composting process proceeded for 24 h at a controlled temperature of 60 °C with a moisture content of 50–60%. Following composting, the material was removed from the machine and left uncovered for 5–7 days until the temperature of the compost pile stabilized at room temperature [11]. The finished compost was then qualitatively characterized according to the Thai Agricultural Standard [12].

2.2 Study of effects of water hyacinth compost on growth of lettuce

Seeds of green oakleaf lettuce (*Lactuca sativa var. crispa* L.) were obtained from a supplier in Hat Yai, Songkhla, Thailand. The seeds were germinated in plastic trays (11 × 21 inches, 200 cells) filled with standard nursery substrate and maintained under controlled nursery conditions for 14 days [13]. After germination, uniform seedlings (approximately 5–7 cm in height) were selected and transplanted into 8-inch plastic pots (capacity: 4 L).

The experiment was conducted using a completely randomized design with 8 treatments and 5 replicates per treatment, totaling 40 pots (experimental units). Each pot was filled with a growing medium based on a mixture of 70% (v/v) topsoil and 30% (v/v) coconut coir. WH compost was added to the base mixture at ratios of 0%, 10%, 30%, 50%, 70%, and 90% (v/v) (Table 1). Two commercial soil brands, purchased from nurseries in Hat Yai, Songkhla, served as controls without WH compost. The pots were randomly arranged in a single experimental area to minimize positional bias. The study was carried out under field conditions from February to April 2024 at the Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Thailand (7° 00′ N, 100° 30′ E). Plants were grown under natural sunlight and protected from excessive rainfall by a transparent roof. Each pot was irrigated daily with 500 mL of tap water to maintain soil moisture at approximately 60–70% of water-holding capacity. Growth parameters were monitored regularly, and plant samples were collected at the end of the growing period for final analysis.

Table 1 Composition of growing media used for green oakleaf lettuce cultivation.

Treatment	Planting material (%)	WH Compost (%)
T1 (Control)	100	0
T2	90	10
T3	70	30
T4	50	50
T5	30	70
T6	10	90
T7	100% commercial soil of brand A	
T8	100% commercial soil of brand B	

Note: The planting material consisted of 70% (v/v) topsoil and 30% (v/v) coconut coir. WH compost refers to water hyacinth compost. Commercial soils (T7 and T8) were purchased from local nurseries in Hat Yai, Songkhla, Thailand, and used without any addition of WH compost.

2.3 Measurements of plant growth

After a cultivation period of 45 days, lettuce plants were harvested and gently rinsed with tap water to remove residual soil and debris. Plant growth was evaluated based on six parameters: shoot length, root length, number of leaves, stem diameter, canopy width, and fresh biomass, measured following the method of Deepika and MubarakAli [14]. Fresh weight was recorded immediately after washing, while dry weight was determined by drying samples in a hot-air oven at 65 °C for 72 h (or until constant weight), following Wongkrachang and Rattaneetoo [15]. The percentage of dry matter was calculated from the ratio of dry to fresh biomass.

2.4 Biochemical analysis

Biochemical analyses were conducted using fresh leaves randomly collected from five plants per treatment. All assays were performed in triplicate, and the average of the replicates was used for subsequent statistical analysis. Chlorophyll and carotenoids: Total chlorophylls and carotenoids were determined from 5 g of fresh leaf tissue homogenized in 15 mL of 99.5% acetone (analytical grade; RCI Labscan, Thailand) and incubated in darkness for 48 hours. The extracts were centrifuged at 5,000 rpm for 15 min, and the absorbance of the supernatant was measured at 470, 645, and 663 nm using a UV–Vis spectrophotometer (Thermo Fisher Scientific, USA). Concentrations of chlorophyll a, chlorophyll b, and carotenoids were calculated according to the following equations [16, 17]:

$$\text{Chlorophyll a} = [12.7 \times OD663 - 2.69 \times OD645] \times V / (1000 \times W) \quad (1)$$

$$\text{Chlorophyll b} = [22.9 \times OD645 - 4.68 \times OD663] \times V / (1000 \times W) \quad (2)$$

$$\text{Carotenoids} = [1000 \times OD470 - 3.27 \times (chlorophyll\ a - chlorophyll\ b)] \times V / (229 \times W). \quad (3)$$

In the above equations, V is the volume of acetone used (L), W is the weight of the sample (g) and OD refers to the optical density at the respective wavelengths.

The carbohydrate content of supernatants was analyzed using the anthrone test. The reagent was prepared by dissolving 0.2 g of anthrone in 100 mL of concentrated sulfuric acid (98%, RCI Labscan, Thailand). Glucose concentrations from 0 to 100 µg/mL were used to generate a standard curve. Subsequently, 5 mL of anthrone reagent were added to the standards and samples, heated in a boiling water bath for 10 min and allowed to cool to room temperature before absorbance was measured at 630 nm [18].

Protein content was determined using the Lowry method. Protein was extracted as for carbohydrate analysis. Approximately 1 g of fresh minced leaf tissue was homogenized and extracted, and 0.1 mL of the collected supernatant was pipetted into a clean test tube. A series of protein standard solutions, usually containing bovine serum albumin (BSA), with known concentrations of 0, 20, 40, 60, 80 and 100 µg/mL were prepared for calibration. Lowry's reagent was prepared by mixing an alkaline copper sulfate solution and 5.0 mL of the reagent were added to each test tube (including standards and samples). The test tubes were shaken with a vortex machine and the mixtures were allowed to stand at room temperature for 10 min. Then, 0.5 mL of Folin–Ciocalteu reagent (previously diluted to 1N) was added to each test tube, mixed immediately, and incubated at room temperature in the dark for 30 min to develop color. The absorbance of each solution was measured at 750 nm with a UV-Vis spectrophotometer. A standard curve was constructed from the absorbance values of the protein standards and the protein concentration of each sample was calculated by comparing its absorbance with the calibration curve [19].

Reducing sugars were quantified using the colorimetric DNS (dinitro-salicylic acid) method [20]. The same extraction method was used as for carbohydrate analysis. Standard glucose solutions (0–1,000 µg/ mL) were prepared and each solution was mixed with DNS reagent in a 1:1 ratio. The mixtures were boiled for 5 min, cooled rapidly in cold water and the absorbance was recorded at 570 nm. The sample extracts were treated in the same way to determine the reducing sugar content from the standard curve [21].

2.5 Statistical analysis

All data were expressed as mean ± standard deviation. The homogeneity of variances was tested using the Levene test, and the normality of the data distribution was assessed using the Shapiro-Wilk test. For data that met the parametric assumptions, a one-way ANOVA was performed, followed by Tukey's HSD test for post-hoc comparisons. In cases where the data did not meet the parametric criteria, the Kruskal-Wallis test was used, with Dunn's test applied for subsequent multiple comparisons. Statistical analyzes were performed using R software (version 4.1.0), with the significance level set at $p < 0.05$.

3. Results and discussion

3.1 Physicochemical properties of water hyacinth compost

All physicochemical properties of the WH compost met the specifications of the compost standards [12] (Table 2). The germination index (GI) was 90.22%, which indicated that the WH compost was fully decomposed and contained no phytotoxic substances, as a GI value above 80% generally indicates a mature and safe compost according to Kong et al. [22]. In addition, heavy metals detected in the WH compost were well below the limits prescribed in the Thai Agricultural Standards [12] and EPA standards [23] for compost. These results showed that the WH compost was safe to be used to promote plant growth, especially for leafy plants as it had a high nitrogen content.

Table 2 Physicochemical properties of water hyacinth compost.

Parameter	Criteria of compost*	Water hyacinth compost	Testing method
pH	Not configured standard	8.04	[24]
Total nitrogen (%w/w)	≥ 1.0	2.53	[25]
Total P_2O_5 (%w/w)	≥ 0.5	1.40	[26]
Total K_2O (%w/w)	≥ 0.5	1.43	[27]
Organic matter, OM (%w/w)	≥ 20	66.31	[28]
C/N Ratio	≤ 20:1	15:1	[29]
Electrical Conductivity, EC (ds/m)	≤ 10	3.42	[30]
Germination index (GI) (%)	≥ 80	90.22	[28,31]
Rock and Gravel, size ≥ 5 mm. (%w/w)	≤ 2	Not detected	[31]
Moisture content(%w/w)	≤ 30	9.18	[32]
Plastic, glass, sharp material and other materials	Not detected	Not detected	[31]
Arsenic (mg/kg)	≤ 50	6.211	[33]
Cadmium (mg/kg)	≤ 5	0.292	[33]
Chromium (mg/kg)	≤ 300	16.29	[33]
Copper (mg/kg)	≤ 500	9.087	[33]
Lead (mg/kg)	≤ 500	1.334	[33]
Mercury (mg/kg)	≤ 2	Not detected	[33]

Note: *Thai Agricultural Standard for compost.

3.2 Effects of water hyacinth compost on the growth of lettuce

The physical growth parameters of green oakleaf lettuce were determined after 45 days in different growth media. The lettuce plants performed best in treatments T2 and T3 (Figure 1). Figure 2 shows that plants grown in T2 exhibited the highest values for plant length (27.33±1.53 cm), root length (18.33±1.53 cm), leaf number (26.83±0.76 leaves), bush width (28.00±1.00 mm), plant thickness (19.23±073 mm), leaf weight (98.29±1.00 g) and dry weight (4.93±0.03 g).

In addition, the presence of WH compost in the planting material significantly influenced all seven growth characteristics. Data analysis by one-way ANOVA showed statistically significant differences between the eight treatment groups for the following parameters: Plant height ($F_{7,16} = 125.57$, $p < 0.0001$), root length ($F_{7,16} = 41.58$, $p < 0.0001$), leaf number ($F_{7,16} = 405.23$, $p < 0.0001$), stem thickness ($F_{7,16} = 1209.88$, $p < 0.0001$), crown width ($F_{7,16} = 388.21$, $p < 0.0001$), fresh biomass ($F_{7,16} = 8993.79$, $p < 0.0001$) and dry biomass ($F_{7,16} = 330.88$, $p < 0.0001$). These differences in plant growth were probably due to the different nutrient profiles provided by the different growing media.

Figure 1 Growth characteristics of lettuce plants in different growing media: T1 (control): 100% planting material (70% top soil mixed with 30% coconut coir by volume), T2-T6: WH compost added to the planting material at 10%, 30%, 50% 70% and 90% (v/v) respectively, T7: commercial soil brand A, T8: commercial soil brand B.

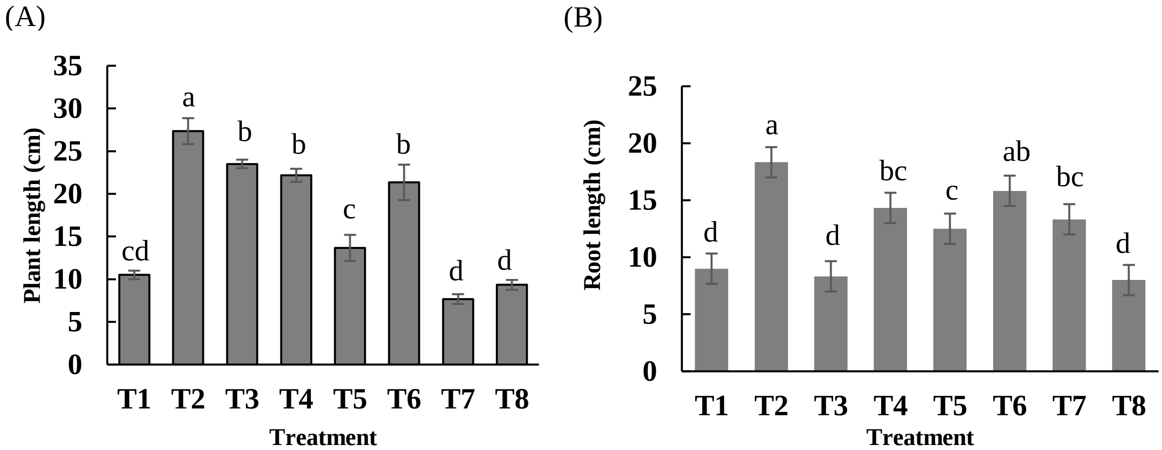

Figure 2 Growth parameters of green oakleaf lettuce plants grown in different growing media for 45 days: T1(control): 100% planting material (70% top soil mixed with 30% coconut coir by volume), T2-T6: WH compost added to the planting material at 10%, 30%, 50% 70% and 90% (v/v) respectively, T7: commercial soil brand A, T8: commercial soil brand B. (A) Chlorophyll a concentration, (B) Chlorophyll b concentration, (C) Carotenoid concentration, (D) Reducing sugar concentration, (E) Protein concentration, (F) Carbohydrate concentration. Vertical bars represent the standard deviation (n=5). Different lowercase letters above the bars denote significant differences among treatments according to Tukey's HSD test ($p < 0.05$).

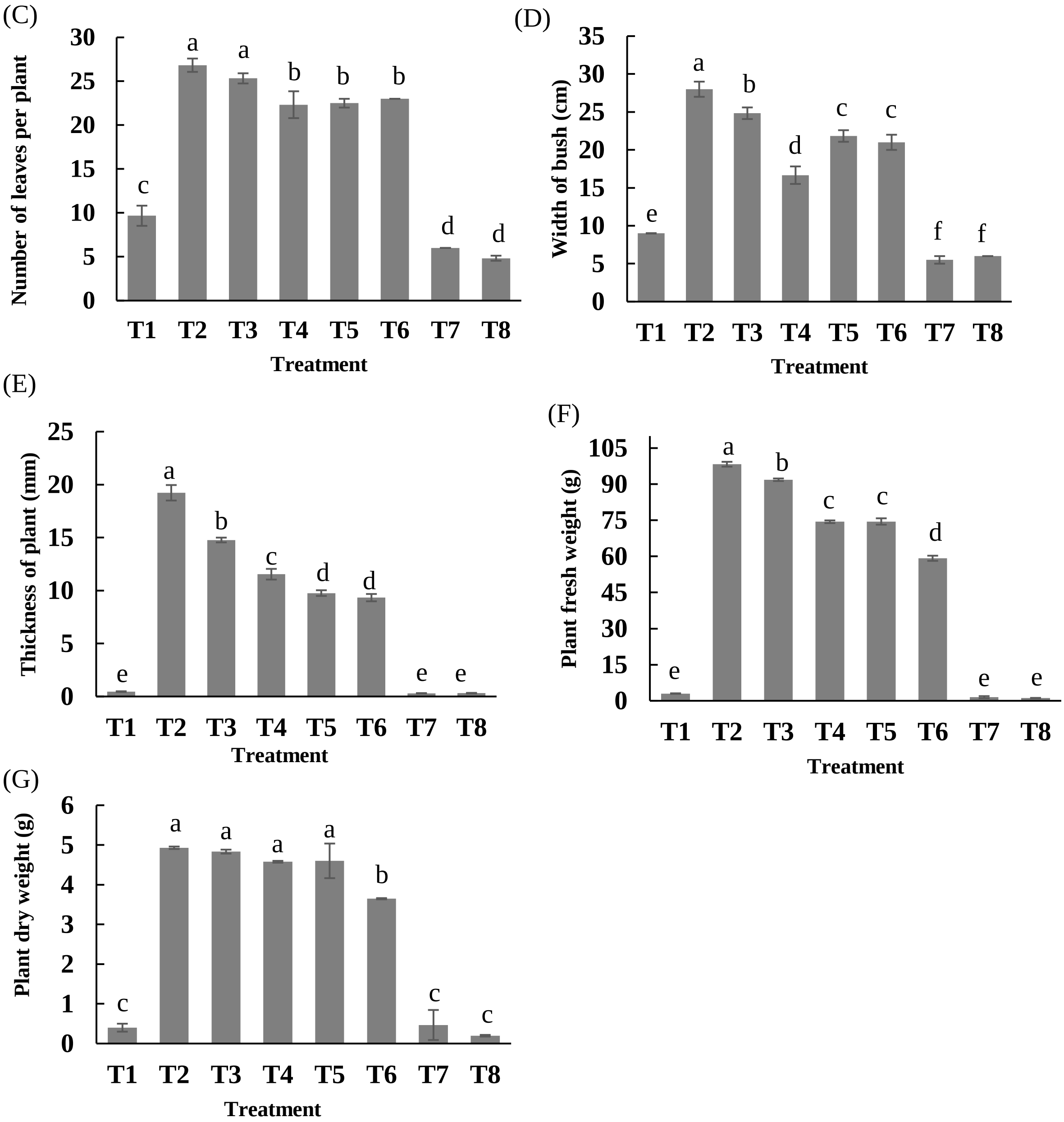

Figure 2 (cont.) Growth parameters of green oakleaf lettuce plants grown in different growing media for 45 days: T1(control): 100% planting material (70% top soil mixed with 30% coconut coir by volume), T2-T6: WH compost added to the planting material at 10%, 30%, 50% 70%, and 90% (v/v) respectively, T7: commercial soil brand A, T8: commercial soil brand B. (A) Chlorophyll a concentration, (B) Chlorophyll b concentration, (C) Carotenoid concentration, (D) Reducing sugar concentration, (E) Protein concentration, (F) Carbohydrate concentration. Vertical bars represent the standard deviation (n=5). Different lowercase letters above the bars denote significant differences among treatments according to Tukey's HSD test ($p < 0.05$).

3.3 Biochemical analysis

Lettuce plants grown in T2 showed the highest values for protein content (488.47±1.36 µg/mL), carbohydrate content (3909.67±1.08 µg/mL) and reducing sugar content (717.70±2.39 µg/mL). The highest levels of chlorophyll a (1.72±0.15 mg/g), chlorophyll b (1.39±0.01 mg/g) and carotenoids (749.47±0.75 mg/g) were found in lettuce plants grown in T6, T5 and T5, respectively (Figure 3). High chlorophyll contents may indicate plant stress caused by changes in endogenous hormone balance [34, 35]. However, plants grown in treatment T6 (90% WH compost) had the highest chlorophyll a content but did not show better growth performance. This outcome could be due to the high alkalinity (pH 8.04) of the WH compost, which can hinder the uptake of essential micronutrients such as phosphorus and iron, and thus impair the physiological functions of plants [13, 21]. In addition, the application of excessive WH compost (as in treatments T5 and T6) led to significant decreases in growth parameters—perhaps due to increased compaction of the substrate, which limits the availability of oxygen to the roots. Yadav et al [37] also found that the excessive use of compost can lead to accumulations of nitrate and ammonium, which can impair photosynthesis and root development and raise food safety concerns for leafy vegetables. These results highlight the importance of optimizing compost ratios to increase plant productivity while avoiding the potential negative effects of nutrient overload or pH imbalance. Biochemical levels were higher in lettuce plants grown with WH compost than without. These results are consistent with previous findings by Muscolo et al [36], who reported that organic fertilizers from plant residues can improve biochemical, carbohydrate and protein synthesis more effectively than non-fertilized soils. Thus, the addition of 10% WH compost mixture to the planting material was the optimal condition for lettuce and resulted in the best growth parameters after 45 days.

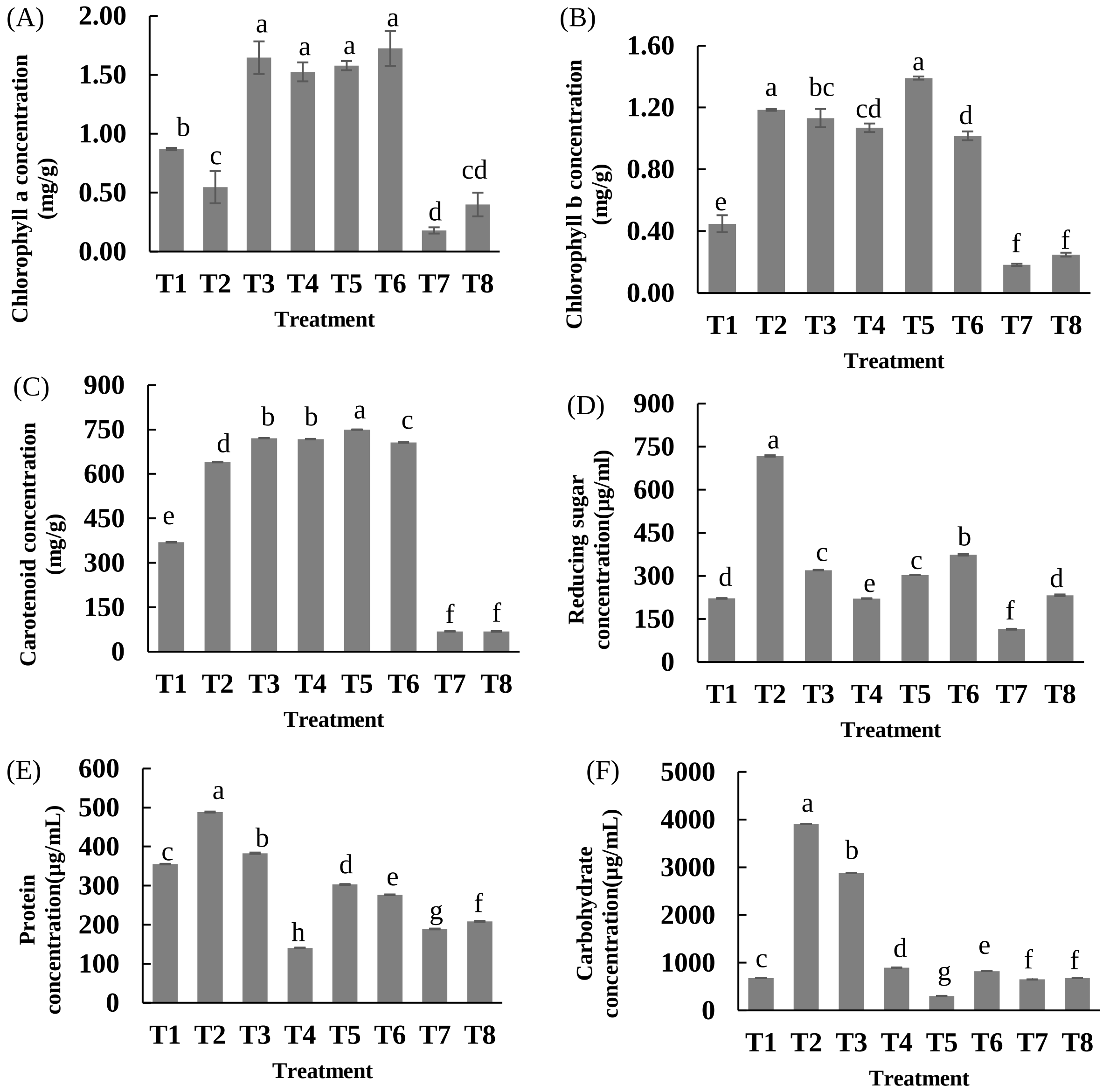

Figure 3 Chlorophyll a, chlorophyll b, total and b, carotenoids reducing sugar, and protein carbohydrate of green oak lettuce plants grown in different growing media for 45 days: T1(control): 100% planting material (70% top soil mixed with 30% coconut coir by volume), T2-T6: WH compost added to the planting material at 10%, 30%, 50% 70% and 90% (v/v) respectively, T7: commercial soil brand A, T8: commercial soil brand B. (A) Chlorophyll a concentration, (B) Chlorophyll b concentration, (C) Carotenoid concentration, (D) Reducing sugar concentration, (E) Protein concentration, (F) Carbohydrate concentration. Vertical bars represent the standard deviation (n=5). Different lowercase letters above the bars indicate significant differences among treatments according to Tukey's HSD test ($p < 0.05$).

4. Conclusions

The physicochemical properties of water hyacinth (WH) compost, including heavy metals, were within the acceptable range according to compost standards. WH compost was added to a base planting mixture in different ratios by volume. The effects of WH compost on the growth of lettuce plants were investigated and compared with the results of growing lettuce in commercial soil media without WH compost. Among the media tested, the mixture with 10% WH compost resulted in the best growth parameters of shoot and root length, leaf number, leaf spread, stem thickness, fresh leaf mass and dry biomass. Contents of protein, carbohydrate and reducing sugar contents were also highest in plants grown with 10% WH compost. These results underline the need for careful evaluation and optimization of compost application rates prior to large-scale agricultural use. WH compost, when properly applied, offers a sustainable, environmentally friendly fertilizer alternative and a promising strategy to mitigate the environmental impact of invasions of this aquatic weed.

5. Author contributions

Jutarut Iewkittayakorn: Conceptualization, Methodology, Data curation, Writing – original draft, Writing – review & editing. Aunkamol Kumngen: Data curation, Writing – original draft, Visualization, Investigation. Usmana Meehae: Visualization, Investigation. Pattarawadee Wongsuwan: Methodology. Siwapong Leunram and Juntima Chungsiriporn: Conceptualization, Methodology, Visualization.

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7. Conflicts of Interest

The authors declare no conflict of interest.

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