

Article

Foliar Application of Hempseed Press Cake-Derived Protein Hydrolysate Enhances Yield, Fruit Quality, and Antioxidant Properties of Strawberry (*Fragaria × ananassa* Duch.) Grown Under Smart Greenhouse Conditions

Bhornchai Harakotr , Tipsuda Teanthong, Lalita Charoensup and Ornprapa Thepsilvisut

Department of Agricultural Technology, Faculty of Science and Technology, Thammasat University, Pathum Thani 12120, Thailand; tipsuda.tea@gmail.com (T.T.); lalita.charao@dome.tu.ac.th (L.C.); ornprapa@tu.ac.th (O.T.)

* Correspondence: harakotr@tu.ac.th; Tel.: +66-2-564-4440 (ext. 2364)

Highlights

What are the main findings?

- Foliar application of hempseed press cake-derived protein hydrolysate improved growth, mineral accumulation, yield, fruit quality, and antioxidant-related traits of strawberry grown under smart greenhouse conditions.
- HPH at 7.5% (*w/v*) provided the best overall response, enhancing fruit set, total yield, fruit size, total soluble solids, phenolic and flavonoid contents, ascorbic acid content, and ABTS antioxidant activity.
- HPH at 10.0% (*w/v*) was more closely associated with selected mineral accumulation, fruit firmness, total anthocyanin content, and DPPH antioxidant activity.

What are the implications of the main findings?

- Hempseed press cake-derived protein hydrolysate may serve as a circular bioeconomy-based foliar biostimulant for sustainable strawberry production.
- The findings provide practical information for improving the productivity and fruit quality of strawberry under smart greenhouse conditions.

Abstract

Hempseed press cake-derived protein hydrolysate (HPH) is a promising plant biostimulant, but its effects on strawberry fruit production under smart greenhouse conditions remain unclear. The objective of this study was to evaluate the effects of foliar HPH application on growth, mineral accumulation, reproductive traits, yield, fruit quality, and antioxidant properties of strawberry cv. ‘Pharachatan 80’. Plants were grown in a smart greenhouse and treated with water (control), a commercial biostimulant, and HPH at 7.5 and 10.0% (*w/v*) (HPH7.5 and HPH10.0, respectively). Foliar application was initiated 25 days after transplanting and repeated weekly for five applications. HPH applications showed clear concentration-specific improvements in vegetative growth, mineral accumulation, reproductive performance, fruit yield, and antioxidant quality. As the most effective treatment for overall crop performance, HPH7.5 enhanced plant growth and mineral accumulation, particularly K and Mg. Consequently, it improved both yield components (fruit set, number, weight, size, and total yield) and fruit quality attributes, such as total soluble solids, total phenolics, flavonoids, ascorbic acid, and ABTS radical-scavenging activity. Conversely, HPH10.0 was more closely associated with N, P, Ca, Fe, Zn, and Mn accumulation, as well as fruit firmness and total anthocyanin content, and DPPH radical-scavenging activity.



Academic Editors: Filippas Bantis, Ioannis Lycoskoufis and Francesco Giuffrida

Received: 29 June 2026

Revised: 22 July 2026

Accepted: 31 July 2026

Published: 2 August 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

Principal component analysis confirmed distinct response patterns between HPH concentrations. Thus, HPH7.5 serves as an effective foliar biostimulant to improve strawberry productivity and quality in smart greenhouses.

Keywords: circular bioeconomy; controlled environment agriculture; fruit quality; plant biostimulant; hempseed press cake; protein hydrolysate; secondary metabolites; strawberry

1. Introduction

The cultivated strawberry (*Fragaria × ananassa* Duch.) is a high-value horticultural crop valued for both market demand and nutritional quality. Global market demand for strawberries continues to expand due to their favorable sensory attributes and high concentrations of bioactive compounds, including phenolics, flavonoids, and anthocyanins, which contribute to fruit color and antioxidant capacity [1–3]. In addition, strawberries are an important source of ascorbic acid, minerals, and dietary fiber, and their regular consumption has been associated with improved antioxidant status and metabolic health [3,4]. Consequently, improving both yield and fruit nutritional quality remains a major targets in modern strawberry production.

To meet increasing year-round demand, strawberry cultivation has progressively shifted toward controlled-environment agriculture (CEA), particularly smart greenhouse systems. These systems enable partial regulation of microclimatic conditions, irrigation, and crop management, thereby improving productivity and reducing the risks associated with climatic variability [1,5]. In tropical and subtropical regions, greenhouse systems are particularly important for moderating high temperatures and excessive solar radiation. Greenhouse covering materials and thermal screen configurations can modify microclimate and influence strawberry yield, while tropical-adapted temperature-control systems have been reported to improve crop physiological performance and quality under warm conditions [6,7]. However, despite these advantages, greenhouse production still faces important constraints. Reduced light transmission through greenhouse structures can limit photosynthetically active radiation (PAR), thereby affecting carbon assimilation and the biosynthesis of light-dependent compounds such as anthocyanins [3]. In strawberry, reproductive development and fruit set are also highly sensitive to greenhouse microclimate, especially temperature and light conditions, which can strongly influence inflorescence development, flower formation, fruit set, and yield potential [8–12]. Moreover, the intensive input requirements of these systems highlight the need for more sustainable strategies to enhance nutrient use efficiency and maintain high fruit quality under controlled growing conditions [13,14].

Plant biostimulants have recently emerged as an alternative to conventional fertilization for improving crop performance. Among these, protein hydrolysates (PHs) are gaining increasing attention for their ability to stimulate plant metabolism and physiological processes. Derived from the hydrolysis of protein-rich materials, PHs contain a mixture of amino acids and bioactive peptides that can modulate key metabolic pathways, including carbon and nitrogen assimilation [15,16]. Amino acid- and peptide-based biostimulants have also been reported to improve photosynthetic performance, nutrient acquisition, and nitrogen use efficiency. Navarro-León et al. [17] demonstrated that L-amino acid-based biostimulants increased lettuce biomass, chlorophyll accumulation, photosynthetic performance, NO_3^- and total N accumulation, and nitrogen use efficiency under different N supplies. In addition to their nutritional contribution, PHs act as signaling molecules with hormone-like effects, promoting root development, enhancing nutrient

uptake, and improving overall plant growth [16,18]. Evidence from strawberry studies indicates that non-microbial biostimulants, including vegetable-derived, alfalfa-based, and amino acid-based formulations, can improve vegetative growth, mineral nutrition, photosynthetic performance, flowering, yield, fruit quality, and resilience under protected or nutrient-limited conditions [19–21]. More recent PH-focused studies in strawberry and other fruit crops also show effects on yield formation, mineral nutrition, and fruit functional quality, although responses depend on PH source, crop species, application mode, and growing conditions [22–24]. Moreover, PHs application has been associated with antioxidant metabolism and fruit functional quality in horticultural crops, including strawberry [18,22,25–28]. This response may be linked partly to amino acid and peptide composition, including phenylalanine, a precursor of the phenylpropanoid pathway involved in the biosynthesis of phenolics, flavonoids, and anthocyanins [29,30].

The valorization of agro-industrial by-products into plant biostimulants is an important strategy in the circular bioeconomy. As a protein-rich by-product of the hemp oil industry, hempseed press cake offers a well-balanced essential amino acid profile and can be valorized as a sustainable raw material for PHs production [25,31]. Such conversion not only reduces underutilized biomass but also generates value-added inputs for sustainable horticultural production. Recent studies have shown that circular-economy-derived biostimulants and waste-based bioprocesses can improve plant growth, nutrient uptake, and fruit quality in greenhouse strawberry and other crops [32,33]. In our previous study, hempseed press cake-derived PH (HPH) improved vegetative growth, mineral contents, and antioxidant-related traits in greenhouse-grown Red Oak lettuce, with 7.5% (*w/v*) identified as an effective concentration [25]. However, comprehensive information on HPH in strawberries remains limited, especially under tropical smart greenhouse conditions. It is also unclear whether the concentration that was previously effective in leafy vegetables can improve reproductive performance, yield formation, and antioxidant-related fruit quality in strawberry. Therefore, the objective of this study was to evaluate the effects of foliar application of HPH on plant growth, mineral accumulation, reproductive traits, yield performance, fruit quality, and antioxidant properties of strawberry cv. ‘Pharachatan 80’ cultivated under a smart greenhouse system.

2. Materials and Methods

2.1. Experimental Conditions, Plant Materials, and Experimental Design

The experiment was conducted from January to March 2026 in a smart greenhouse at the Agricultural Technology Farming Center, Faculty of Science and Technology, Thammasat University, Pathum Thani, Thailand (14.07450° N, 100.6094167° E; 7.3 m a.s.l.). Greenhouse microclimate was monitored throughout the cultivation period. The roof was fitted with multifunctional plastic film (MultiTech, MTEC, NSTDA, Pathum Thani, Thailand), a cladding material designed for high ultraviolet filtration and high near-infrared (NIR) reflectance. This film was used to reduce incoming radiation and heat load during production under tropical greenhouse conditions. Air temperature and relative humidity were logged using SHT31 sensors (Tony Space, Bangkok, Thailand), whereas photosynthetically active radiation (PAR) was recorded using custom-developed phototransistor sensors from the Thai Microelectronics Center (TMEC, Mueang District, Chachoengsao, Thailand), following the sensor configuration described by Kumsong et al. [7]. During the experimental period, the greenhouse reduced the mean maximum air temperature from 41.52 °C outside to 35.92 °C inside. Mean minimum temperature was similar between environments, with a slightly higher value inside the greenhouse than outside (24.72 vs. 24.02 °C). Minimum relative humidity increased from 36.88% outside to 47.16% inside, while PAR decreased from 1581.86 to 290.84 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure S1).

Strawberry (*Fragaria* × *ananassa* Duch.) cv. ‘Pharachatan 80’ runner plants were obtained from a commercial nursery in Chiang Mai, Thailand. This cultivar was developed for cultivation under Thai highland conditions and is characterized by its adaptability to warm environments, large fruit size, pleasant aroma, sweet flavor, and high yield potential. Uniform 30-day-old plantlets were selected and transplanted on 15 January 2026 into growing bags (20 cm in diameter) filled with a commercial growing medium from the same source as previously reported by Harakotr et al. [25] and cultivated under greenhouse conditions. The reported chemical properties of this growing medium were pH 6.39, EC 3.00 dS m⁻¹, organic matter 35.13%, total N 1.31%, total P 2.27%, total K 1.32%, total Ca 1.59%, total Mg 0.53%, and C/N ratio 15.58. Before planting, a 15–15–15 compound fertilizer was applied at a rate of 187.5 kg ha⁻¹, which supplied 28.13–28.13–28.13 kg ha⁻¹ of N–P₂O₅–K₂O. At the fruit-setting stage, 13–13–21 compound fertilizer was applied at the same rate, equivalent to 24.38–24.38–39.38 kg ha⁻¹ of N–P₂O₅–K₂O. Pests and diseases were managed according to the standard recommendations of the Department of Agriculture, Thailand.

The trial followed a completely randomized design (CRD) with three replications. Each replication included 10 plants per treatment, resulting in 30 plants per treatment. Four treatments were applied as follows: (i) Control: distilled water spray; (ii) Commercial biostimulant (CB): All bio-organic plant growth booster biostimulant, distributed by Supreme Bio Trading Ltd. (Bangkok, Thailand), with the amino acid compositions of CB and HPH provided in Table S1; (iii) HPH7.5: carrier formulation supplemented with 7.5% (*w/v*) HPH; (iv) HPH10.0: carrier formulation supplemented with 10.0% (*w/v*) HPH. The HPH treatments were prepared in-house using a carrier formulation containing stabilizing agents, preservatives, and micronutrients, following the formulation previously reported by Harakotr et al. [25]. Deionized water was used to prepare the final foliar spraying solutions, and their pH and EC values are presented in Table S2. Foliar spraying began on 19 February 2026, corresponding to 25 days after transplanting (DAT) and the late vegetative to early flowering stage, before full flowering. Foliar spraying was conducted in the evening using a 2 L hand-pressure sprayer, with 100 mL of the assigned solution applied per plant to uniformly wet both adaxial and abaxial leaf surfaces without runoff. The application was repeated every 7 days for five applications (Figure 1).

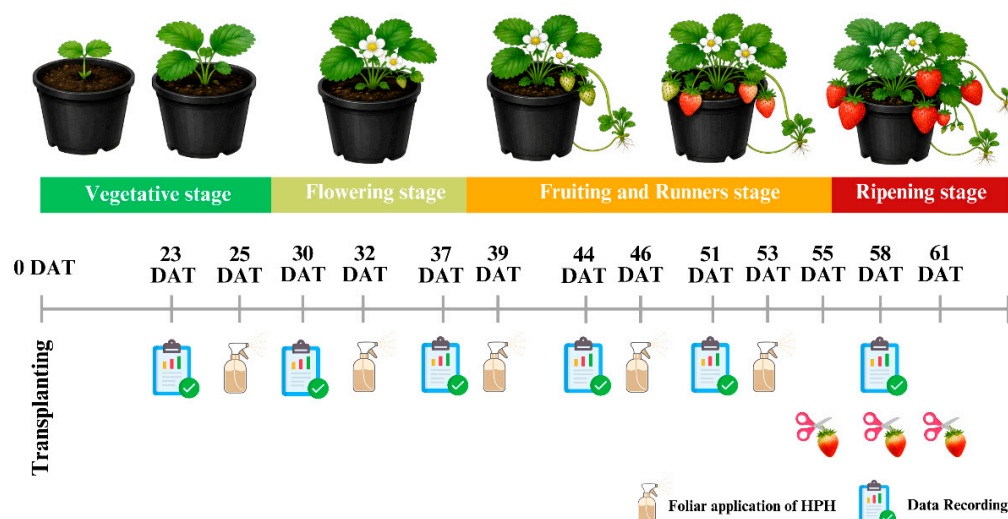


Figure 1. Experimental timeline for foliar hempseed press cake-derived protein hydrolysate (HPH) application in strawberry cv. ‘Pharachatan 80’, showing the five spraying events at 25, 32, 39, 46, and 53 days after transplanting (DAT).

2.2. Growth, Reproductive, Yield, and Quality Parameters

Vegetative growth was monitored at six sampling dates during the experimental period. Plant height (PH; cm) was measured at 23, 30, 37, 44, 51, and 58 DAT from the base of the plant to the tip of the longest leaf. Plant width (PW; cm) was determined at the same intervals by measuring the maximum horizontal canopy spread. The numbers of runners (NR) and leaves (NL) were recorded per plant and expressed as runners plant^{−1} and leaves plant^{−1}, respectively.

Reproductive development was evaluated from flowering to harvest by counting the number of inflorescences plant^{−1} (NFP), number of flowers inflorescence^{−1} (NFI), and number of fruits inflorescence^{−1} (NFrI). Measurements were made across successive flowering flushes until the end of the harvest period. Fruit set percentage (FrS) was calculated for each inflorescence from the proportion of retained fruits relative to the total number of flowers:

$$\text{FrS (\%)} = (\text{number of fruits retained inflorescence}^{-1} / \text{total number of flowers inflorescence}^{-1}) \times 100 \quad (1)$$

Fruit set values were then averaged across sampled plants within each treatment.

Strawberry fruits were harvested three times at 3-day intervals, from approximately 55 to 61 DAT, when 70–75% of the fruit surface had developed red coloration. At each harvest, all fruits harvested from each plant were counted and weighed using a digital scale. Total fruit number plant^{−1} (TFrN) and total yield (TY; g FW plant^{−1}) were calculated by summing values across the three harvests. Average fruit fresh weight (FrW; g FW), fruit length (FrL; cm), and fruit width (FrWi; cm) were determined from four representative fruits per plant collected at an intermediate harvest during the production period. FrW was calculated as the mean fresh weight of these selected fruits. The same fruit samples were used for the measurement of external fruit size and basic quality attributes. For biochemical assays, a separate fruit sample set was collected at the same harvest stage, immediately frozen in liquid nitrogen, and freeze-dried. After drying, samples were milled into a fine powder using a variable-speed rotor mill (Pulverisette 14, Fritsch, Idar-Oberstein, Germany) and stored at −20 °C until analysis.

Fruit firmness (FrF) and total soluble solids (TSS) were determined from four fruits per plant. FrF (kg cm^{−2}) was measured using a texture analyzer fitted with a 6 mm diameter probe (Stable Micro Systems, model TA-XT plus, Godalming GU7 1YL, UK). The probe was driven at a test speed of 1.5 mm s^{−1} to a penetration depth of 5 mm. TSS (°Brix) was determined using a portable refractometer (ATAGO, model PAL-1, Tokyo, Japan).

2.3. Determination of Shoot Mineral Content

For mineral analysis, aboveground shoot tissues were sampled at 58 DAT from each treatment. Fresh plant materials were first washed with tap water and then rinsed with deionized water to remove dust and surface contaminants. The cleaned samples were oven-dried to constant weight and subsequently milled to obtain a homogeneous powder. The powdered samples were subjected to wet acid digestion using a mixture of HNO₃ and HClO₄ (6:1, v/v) prior to mineral determination. Total N was determined by the Kjeldahl method, and P was determined colorimetrically using the Vanadomolybdate method, whereas K, Ca, Mg, Fe, Zn, and Mn were measured using atomic absorption spectrophotometry (AAS; Agilent Technologies, Inc., Agilent 200 Series, Santa Clara, CA, USA). All mineral analyses were conducted by the Soil Science Laboratory, Department of Plant and Soil Sciences, Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand. Total N, P, and K were reported as percentages on a dry weight basis, whereas Ca, Mg, Fe, Zn, and Mn were reported as mg kg^{−1} DW, following the original plant nutrient analysis report issued by the analytical laboratory.

2.4. Fruit Bioactive Compounds and Antioxidant Activity

2.4.1. Sample Preparation and Extraction

Freeze-dried strawberry fruit powder was used for ethanol extraction following the approach of Jirakiattikul et al. [34], with slight modifications. For each sample, 5 g of ground material was extracted with 95% ethanol at a sample-to-solvent ratio of 1:3 (*w/v*). The extraction was carried out for 72 h and repeated three times to improve the recovery of ethanol-soluble compounds. After each extraction, the suspension was filtered through filter paper, and the filtrates from the three extraction cycles were combined. The pooled extract was dried at 50 °C for 72 h to obtain a crude dried extract, which was then stored at −20 °C until subsequent analysis. This extract was used for the determination of bioactive compounds and antioxidant activity.

2.4.2. Determination of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

TPC was quantified using a Folin–Ciocalteu-based colorimetric assay adapted from Folin and Ciocalteu [35]. For each reaction, 20 µL of fruit extract was combined with 1.58 mL of distilled water, followed by the addition of 100 µL of Folin–Ciocalteu reagent. After 5 min of reaction at room temperature, 300 µL of 20% Na₂CO₃ was added to develop the color reaction. The reaction mixture was then kept in the dark for 2 h, and absorbance was recorded at 765 nm using a microplate reader (EnVision XCite 2105, Revvity, Waltham, MA, USA). Gallic acid was used to construct the calibration curve, and TPC was expressed as mg gallic acid equivalents g^{−1} dry extract (mg GAE g^{−1} DE).

TFC was determined using the aluminum chloride colorimetric method described by Kubola et al. [36], with minor modifications. Briefly, 0.5 mL of extract was diluted with 2.2 mL of distilled water, after which 0.15 mL of 5% NaNO₂ was added. The mixture was allowed to stand for 6 min before adding 0.3 mL of 10% AlCl₃·6H₂O. After 5 min, 1.0 mL of 1 M NaOH was added to terminate the reaction and stabilize the color. Absorbance was measured at 510 nm using a microplate reader. Quercetin was used as the reference standard, and TFC was expressed as mg quercetin equivalents g^{−1} dry extract (mg QE g^{−1} DE).

2.4.3. Determination of Total Anthocyanin Content (TAC)

TAC was determined by the pH differential method of Lee et al. [37], with minor modifications. For each sample, two aliquots of extract (200 µL each) were diluted separately with 800 µL of potassium chloride buffer (0.025 M, pH 1.0) and sodium acetate buffer (0.4 M, pH 4.5). After equilibration, absorbance was measured at 520 nm and 700 nm for both pH conditions using a UV–Vis spectrophotometer (UV–1280, Shimadzu, Kyoto, Japan). The absorbance difference was calculated as follows:

$$A = (A_{520} - A_{700})_{\text{pH 1.0}} - (A_{520} - A_{700})_{\text{pH 4.5}} \quad (2)$$

TAC was calculated using the Beer–Lambert equation based on a molar absorptivity of 26,900 L mol^{−1} cm^{−1} and a molecular weight of 449.2 g mol^{−1}. The results were expressed as mg cyanidin-3-glucoside equivalents g^{−1} dry extract (mg C3G g^{−1} DE).

2.4.4. Determination of Total Ascorbic Acid Content (TAsA)

TAsA was determined following Bassi et al. [38], with minor modifications. Crude dried extract (0.1 g) was dissolved in 0.2 mL of 5% metaphosphoric acid and reacted with 0.02% indophenol solution. After 3 min, 2% thiourea and 2% 2,4-dinitrophenylhydrazine were added to the reaction mixture. The mixture was heated at 50 °C for 70 min and then allowed to cool to room temperature. Subsequently, 85% sulfuric acid was added, and the reaction was left to develop for 30 min. Absorbance was measured at 540 nm using

a UV-Vis spectrophotometer. TAsA was calculated using an L-ascorbic acid calibration curve and expressed as mg g^{-1} dry extract (mg g^{-1} DE).

2.4.5. Antioxidant Activity

The antioxidant capacity of the strawberry fruit extracts was assessed using DPPH and ABTS radical-scavenging assays. Trolox was used to generate calibration curves for both assays. The DPPH assay was performed according to Brand-Williams et al. [39], with minor modifications. For the reaction, 200 μL of sample extract was added to 1.80 mL of 0.06 mM DPPH solution. After mixing, the reaction mixture was kept in the dark at room temperature for 30 min. Absorbance was then measured at 520 nm using a microplate reader.

The ABTS assay followed the procedure of Re et al. [40], with minor modifications. The ABTS radical cation was generated by combining equal volumes of 14 mM ABTS solution and 4.9 mM potassium persulfate solution, followed by incubation in the dark at room temperature for 16 h. Before use, the ABTS working solution was diluted with ethanol to an absorbance of 0.700 ± 0.020 at 734 nm. For measurement, 200 μL of sample extract was reacted with 1.80 mL of the diluted ABTS solution for 6 min in the dark, and absorbance was measured at 734 nm. Antioxidant activity obtained from both assays was expressed as μmol Trolox equivalents g^{-1} dry extract ($\mu\text{mol TE g}^{-1}$ DE).

2.5. Statistical Analysis

Statistical analyses were conducted using Statistix 10.0 (Analytical Software, Tallahassee, FL, USA). Data were analyzed according to a CRD using one-way analysis of variance (ANOVA), with treatment as the main factor. For growth parameters measured at different sampling dates, ANOVA was performed separately for each date. Treatment means were compared using Tukey's honestly significant difference (HSD) test. Differences were considered statistically significant at $p \leq 0.05$. PCA was performed using R software (R Core Team, version 4.5.1; Vienna, Austria).

3. Results

3.1. Growth of Strawberry cv. 'Pharachatan 80'

The visual response of greenhouse-grown strawberry plants to the foliar application of HPH is shown in Figure 2. Treatment effects on vegetative growth were most evident from 44 to 58 DAT (Figure 3). Compared with control, HPH7.5 and HPH10.0 increased PH, PW, NL, PL, and LW (Figure 3a–e), with responses generally similar to the CB. HPH7.5 showed a consistent increase in PH, LW, and runner production, whereas HPH10.0 produced the widest canopy at 58 DAT.

HPH application also affected runner production from 30 to 58 DAT (Figure 3f). No runners were observed at 23 DAT, but NR increased thereafter. At the later sampling dates, HPH7.5 and the CB produced higher NR than the untreated control. These results indicate that HPH7.5 was the most consistent treatment for improving vegetative growth and runner formation in greenhouse-grown strawberry plants.

3.2. Mineral Composition of Plant Shoots of Strawberry cv. 'Pharachatan 80'

Foliar application of HPH significantly affected mineral accumulation in shoots of strawberry cv. 'Pharachatan 80' at 58 DAT (Table 1). Compared with the untreated control, HPH7.5 increased shoot mineral contents by 4.6–26.8%, with the greatest increase observed for Mg (26.8%) and the smallest for Zn (4.6%). HPH10.0 increased P, Ca, Mg, Fe, Zn, and Mn contents by 10.1–175.3% compared with the control, while N increased by 5.9%. In contrast, K content was slightly lower than the control. Among the treatments, HPH10.0

produced the highest N, P, Ca, Fe, and Zn contents, whereas HPH7.5 resulted in the highest K, Mg, and Mn contents. In contrast, the commercial biostimulant showed lower N, P, Fe, Zn, and Mn contents than the control, although it increased K, Ca, and Mg contents. These results indicate that foliar application of HPH improved mineral accumulation in strawberry shoots, with HPH10.0 being more effective for N, P, Ca, Fe, and Zn, while HPH7.5 was more closely associated with higher K, Mg, and Mn contents.

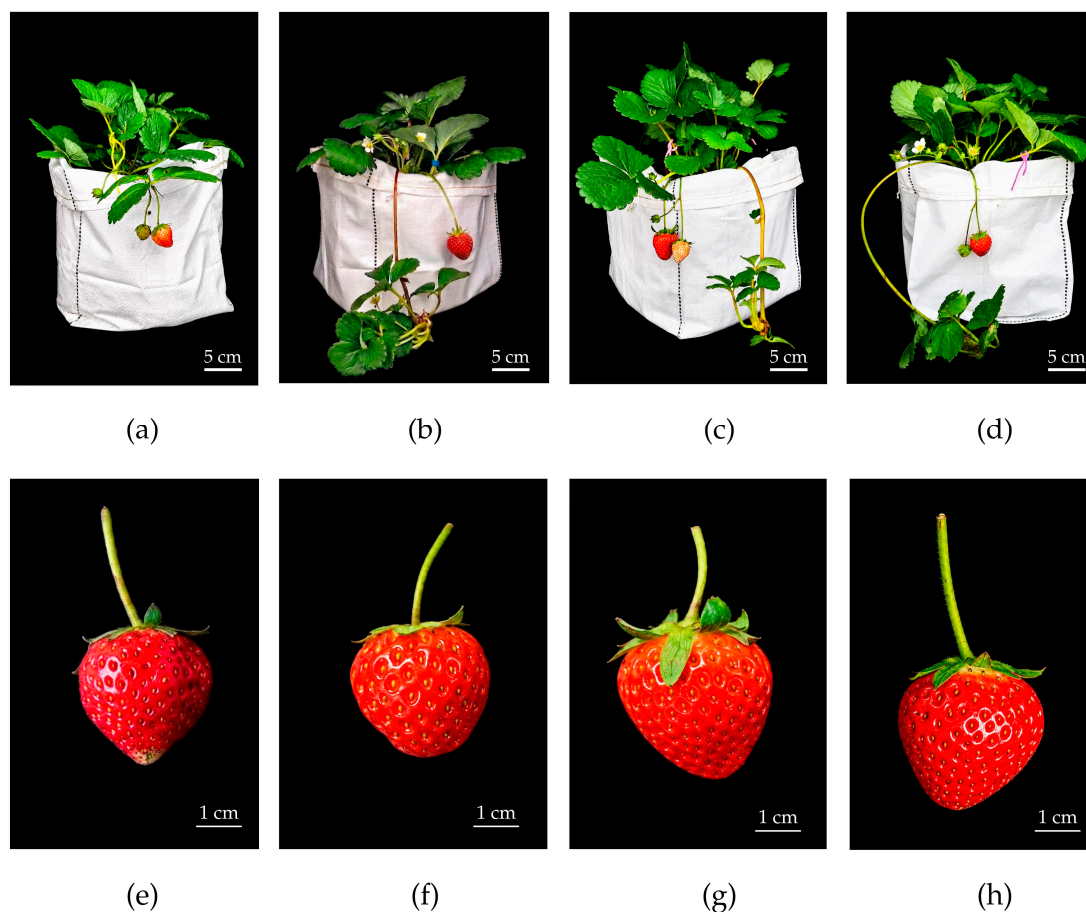


Figure 2. Representative appearance of strawberry cv. ‘Pharachatan 80’ plants and fruit grown under greenhouse conditions, as influenced by foliar application of hempseed press cake-derived protein hydrolysate (HPH). (a,e) Control: sprayed with distilled water; (b,f) CB: commercial biostimulant; (c,g) HPH7.5: carrier formulation supplemented with 7.5% (*w/v*) HPH; and (d,h) HPH10.0: carrier formulation supplemented with 10.0% (*w/v*) HPH.

Table 1. Effect of foliar application of HPH on the mineral contents of strawberry cv. ‘Pharachatan 80’ grown under smart greenhouse conditions at 58 DAT.

Treatments ¹	N (% DW)	P (% DW)	K (% DW)	Ca (mg kg ^{−1} DW)	Mg (mg kg ^{−1} DW)	Fe (mg kg ^{−1} DW)	Zn (mg kg ^{−1} DW)	Mn (mg kg ^{−1} DW)
Control	2.02 ± 0.01 ^{b2}	0.39 ± 0.02 ^c	1.70 ± 0.02 ^c	1.47 ± 0.01 ^d	0.41 ± 0.00 ^d	6.51 ± 0.17 ^c	302.44 ± 2.66 ^b	38.69 ± 0.73 ^c
CB	1.73 ± 0.02 ^c	0.25 ± 0.03 ^d	2.00 ± 0.01 ^b	1.53 ± 0.01 ^c	0.45 ± 0.01 ^c	4.59 ± 0.11 ^d	157.64 ± 3.15 ^c	31.60 ± 0.04 ^d
HPH7.5	2.13 ± 0.00 ^a	0.45 ± 0.01 ^b	2.14 ± 0.02 ^a	1.58 ± 0.02 ^b	0.52 ± 0.01 ^a	7.39 ± 0.10 ^b	316.49 ± 10.80 ^b	44.62 ± 1.23 ^a
HPH10.0	2.14 ± 0.03 ^a	0.53 ± 0.01 ^a	1.68 ± 0.01 ^c	1.65 ± 0.01 ^a	0.50 ± 0.01 ^b	17.92 ± 0.42 ^a	373.45 ± 2.56 ^a	42.58 ± 0.66 ^b

¹ Control: distilled water spray; CB: commercial biostimulant; HPH7.5: carrier formulation supplemented with 7.5% (*w/v*) HPH; HPH10.0: carrier formulation supplemented with 10.0% (*w/v*) HPH. ² Means ± standard deviations followed by the same letters in the same column are not significantly different at $p \leq 0.05$, as determined by Tukey’s HSD test.

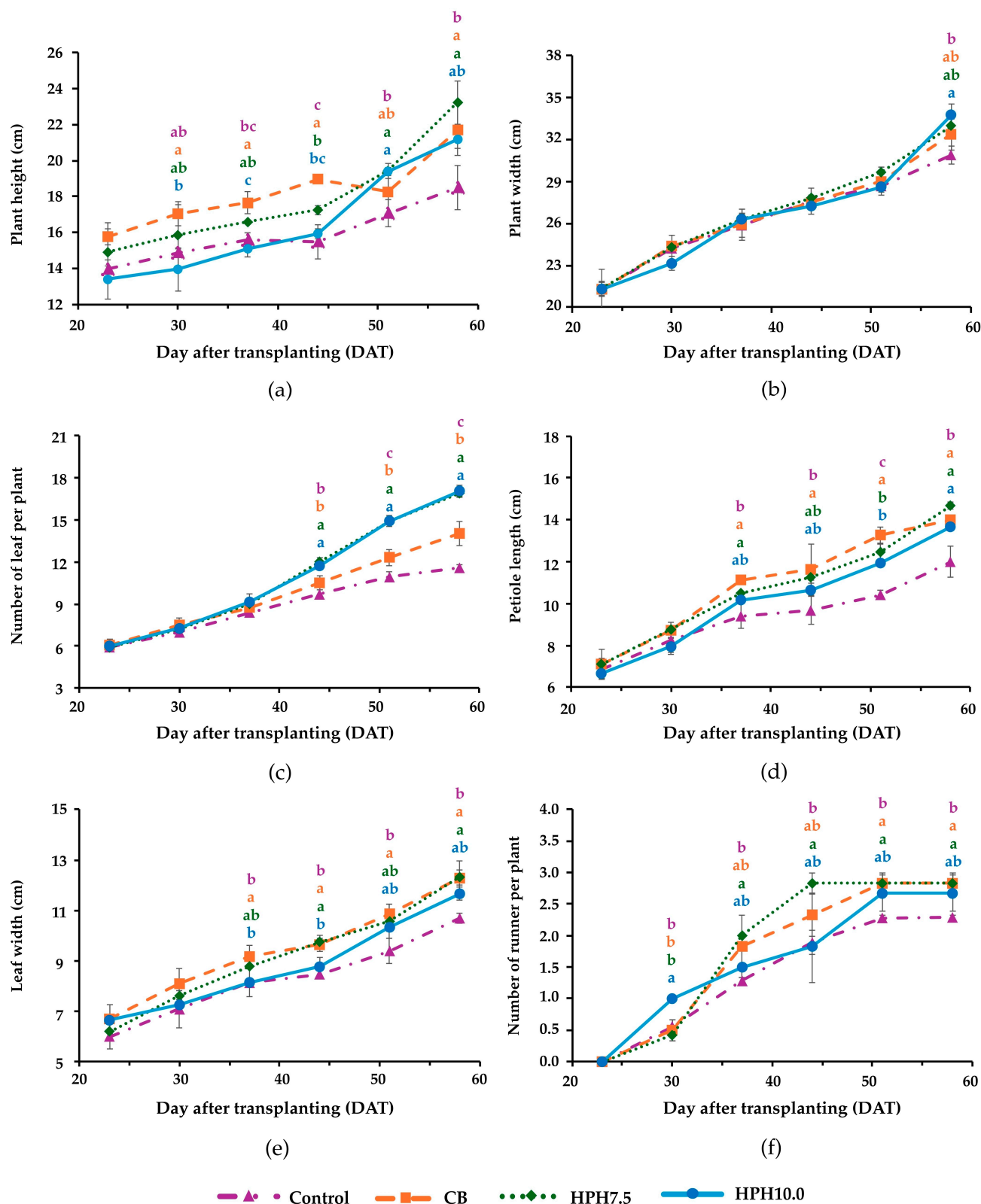


Figure 3. Growth performance of strawberry cv. 'Pharachatan 80' grown under smart greenhouse conditions between 23 and 58 DAT, as influenced by the foliar application of HPH. (a) plant height; (b) plant width; (c) number of leaves plant⁻¹; (d) petiole length; (e) leaf width; (f) number of runners plant⁻¹. Control: distilled water spray; CB: commercial biostimulant; HPH7.5: carrier formulation supplemented with 7.5% (w/v) HPH; HPH10.0: carrier formulation supplemented with 10.0% (w/v) HPH. Values are presented as mean $\pm$ SE ($n = 3$). Different letters indicate significant differences among treatments according to Tukey's HSD test at $p \leq 0.05$.

3.3. Effect of Foliar Application of HPH on Reproductive Traits and Fruit Set of Strawberry cv. ‘Pharachatan 80’

Foliar application of HPH significantly affected the reproductive traits and fruit set of strawberry grown under smart greenhouse conditions (Table 2). HPH7.5 produced the highest NFP, NFrI, and FrS, with values of 4.50 inflorescences plant^{−1}, 4.80 fruits inflorescence^{−1}, and 56.61%, respectively. These values were significantly higher than those of control, which showed the lowest NFP, NFrI, and FrS. In contrast, HPH10.0 produced the highest NFI (9.58 flowers inflorescence^{−1}), although this did not translate into the highest fruit set. Finally, HPH7.5 was the most effective treatment for improving reproductive performance and fruit set.

Table 2. Effect of foliar application of the HPH on reproductive traits and fruit set of strawberry cv. ‘Pharachatan 80’ grown under smart greenhouse conditions.

Treatments ¹	NFP ² (Inflorescences Plant ^{−1})	NFI (Flowers Inflorescence ^{−1})	NFrI (Fruits Inflorescence ^{−1})	FrS (%)
Control	2.17 ± 0.29 ^{c3}	8.25 ± 0.00 ^b	2.33 ± 0.38 ^c	25.76 ± 1.52 ^d
CB	3.33 ± 0.58 ^b	9.00 ± 0.66 ^{ab}	4.39 ± 0.53 ^{ab}	46.62 ± 0.16 ^b
HPH7.5	4.50 ± 0.50 ^a	7.97 ± 0.56 ^b	4.80 ± 0.26 ^a	56.61 ± 2.06 ^a
HPH10.0	3.17 ± 0.29 ^{bc}	9.58 ± 0.14 ^a	3.53 ± 0.31 ^b	38.54 ± 0.93 ^c

¹ Control: distilled water spray; CB: commercial biostimulant; HPH7.5: carrier formulation supplemented with 7.5% (w/v) HPH; HPH10.0: carrier formulation supplemented with 10.0% (w/v) HPH. ² NFP: number of inflorescences plant^{−1}; NFI: number of flower inflorescences^{−1}; NFrI: number of fruit inflorescences^{−1}; FrS: fruit set percentage. ³ Means ± standard deviations followed by the same letters in the same column are not significantly different at $p \leq 0.05$, as determined by Tukey’s HSD test.

3.4. Yield Components and Fruit Quality of Strawberry cv. ‘Pharachatan 80’

Foliar application of HPH significantly affected yield, yield components, and fruit quality of greenhouse-grown strawberries (Table 3). The control had the lowest values for all yield-related traits. In contrast, HPH7.5 produced the highest TY, TFrN, and FrW. Compared with the control, HPH7.5 increased TY by 161.7%, TFrN by 121.1%, and FrW by 141.5%. HPH10.0 also increased these traits by 107.3, 52.4, and 86.8%, respectively.

Table 3. Effect of foliar application of HPH on the yield components and fruit quality of strawberry cv. ‘Pharachatan 80’ grown under smart greenhouse conditions.

Treatments ¹	TFrN ² (Fruits Plant ^{−1})	TY (g Plant ^{−1} FW)	FrW (g FW)	FrL (cm)	FrWi (cm)	FrF (kg cm ^{−2})	TSS (° Brix)
Control	4.92 ± 1.18 ^{c3}	27.07 ± 1.04 ^d	4.31 ± 0.41 ^c	1.94 ± 0.01 ^c	1.93 ± 0.03 ^b	0.54 ± 0.00 ^d	6.60 ± 0.13 ^d
CB	9.03 ± 0.53 ^{ab}	50.91 ± 0.11 ^c	6.29 ± 1.27 ^{bc}	2.12 ± 0.11 ^{bc}	2.29 ± 0.15 ^a	0.88 ± 0.04 ^c	10.51 ± 0.39 ^b
HPH7.5	10.88 ± 0.37 ^a	70.84 ± 2.38 ^a	10.41 ± 0.27 ^a	3.03 ± 0.38 ^a	2.59 ± 0.14 ^a	0.95 ± 0.02 ^b	12.90 ± 0.87 ^a
HPH10.0	7.50 ± 0.50 ^b	56.12 ± 0.79 ^b	8.05 ± 0.76 ^b	2.73 ± 0.30 ^{ab}	2.46 ± 0.12 ^a	1.05 ± 0.02 ^a	8.97 ± 0.55 ^c

¹ Control: distilled water spray; CB: commercial biostimulant; HPH7.5: carrier formulation supplemented with 7.5% (w/v) HPH; HPH10.0: carrier formulation supplemented with 10.0% (w/v) HPH. ² TFrN: total fruit number plant^{−1}; TY: total yield; FrW: average fruit fresh weight; FrL: fruit length; FrWi: fruit width; FrF: fruit firmness; TSS: total soluble solids. ³ Means ± standard deviations followed by the same letters in the same column are not significantly different at $p \leq 0.05$ as determined by Tukey’s HSD test.

For fruit quality, HPH7.5 produced the greatest FrL and TSS, whereas HPH10.0 produced the highest FrF. Compared with the control, HPH7.5 increased FrL, FrWi, FrF, and TSS by 56.2, 34.2, 75.9, and 95.5%, respectively. The corresponding increases under HPH10.0 were 40.7, 27.5, 94.4, and 35.9%, respectively. These results show that HPH7.5 was the most effective treatment for improving the yield and fruit quality of strawberries under smart greenhouse conditions.

3.5. Fruit Bioactive Compounds and Antioxidant Activity of Strawberry cv. 'Pharachatan 80'

Foliar application of HPH significantly affected fruit bioactive compounds and antioxidant activity in strawberry cv. 'Pharachatan 80' grown under smart greenhouse conditions (Table 4). HPH7.5 produced the highest TPC and TFC, with increases of 64.8% and 81.8%, respectively, compared with untreated control. HPH10.0 also increased TPC and TFC by 54.2 and 46.7%, respectively, but these values were lower than those observed under HPH7.5. For TAC, HPH10.0 produced the highest value, increasing TAC by 56.2% compared with the control, whereas HPH7.5 and the CB showed lower values than HPH10.0 but remained significantly higher than the control. TAsA was also enhanced by foliar treatments, with CB, HPH7.5, and HPH10.0 showing significantly higher values than the control. Among the HPH treatments, HPH7.5 and HPH10.0 increased TAsA by 128.2 and 101.6%, respectively. Antioxidant activity also varied among treatments. HPH7.5 showed the highest ABTS activity, representing a 197.3% increase over the control, followed by HPH10.0 and CB. In contrast, HPH10.0 produced the highest DPPH activity, increasing it by 91.0% compared with the control. These results indicate that HPH7.5 was more effective in enhancing TPC, TFC, TAsA, and ABTS activity, whereas HPH10.0 was more closely associated with TAC accumulation and DPPH activity.

Table 4. Effect of foliar application of HPH on bioactive compounds and their antioxidant activities in fruits of strawberry cv. 'Pharachatan 80' grown under smart greenhouse conditions.

Treatments ¹	TPC ² (mg GAE g ⁻¹ DE)	TFC (mg QE g ⁻¹ DE)	TAC (mg C3G g ⁻¹ DE)	TAsA (mg g ⁻¹ DE)	Antioxidant Activities (μmol TE g ⁻¹ DE)	
					ABTS	DPPH
Control	35.13 ± 1.15 ^{d3}	38.04 ± 0.86 ^d	2.67 ± 0.12 ^c	3.08 ± 0.02 ^b	215.43 ± 6.79 ^d	56.86 ± 11.27 ^c
CB	50.16 ± 1.92 ^c	45.28 ± 1.51 ^c	2.99 ± 0.06 ^b	6.09 ± 0.45 ^a	454.32 ± 19.87 ^c	84.54 ± 4.28 ^b
HPH7.5	57.89 ± 0.08 ^a	69.16 ± 0.79 ^a	3.22 ± 0.10 ^b	7.03 ± 0.04 ^a	640.43 ± 18.21 ^a	91.43 ± 1.29 ^b
HPH10.0	54.18 ± 1.07 ^b	55.80 ± 0.59 ^b	4.17 ± 0.15 ^a	6.21 ± 0.73 ^a	510.49 ± 9.26 ^b	108.64 ± 2.82 ^a

¹ Control: distilled water spray; CB: commercial biostimulant; HPH7.5: carrier formulation supplemented with 7.5% (w/v) HPH; HPH10.0: carrier formulation supplemented with 10.0% (w/v) HPH. ² TPC: total phenolic content; TFC: total flavonoid content; TAC: total anthocyanin content; TAsA: total ascorbic acid content; ABTS: ABTS antioxidant activity; DPPH: DPPH antioxidant activity. ³ Means ± standard deviations followed by the same letters in the same column are not significantly different at $p \leq 0.05$, as determined by Tukey's HSD test.

3.6. Principal Component Analysis

PCA was performed to evaluate the relationship among growth traits and mineral contents of strawberry plants under different foliar treatments. The first two principal components explained 85.5% of the total variance, with PC1 and PC2 accounting for 50.5 and 35.0%, respectively (Figure 4a,b). The score plot showed clear treatment separation, with the control and CB located on the positive side of PC1, while HPH7.5 and HPH10.0 were positioned on the negative side. HPH7.5 was separated from HPH10.0 along PC2, indicating different responses between the two HPH concentrations. The loading plot showed that most growth and mineral traits were negatively associated with PC1. HPH7.5 was closely related to vegetative growth traits, including PH, PL, LW, NR, PW, and NL, as well as K and Mg contents. In contrast, HPH10.0 was more associated with N, P, Fe, Zn, Mn, and Ca contents. These results suggest that HPH7.5 mainly enhanced vegetative growth, whereas HPH10.0 was more closely related to mineral accumulation.

For the yield components and fruit quality traits of strawberry cv. 'Pharachatan 80', PC1 and PC2 explained 72.4% and 12.4% of the total variance, respectively (Figure 5a,b). The score plot showed separation among treatments along PC1, with the control located on the negative side, while HPH7.5 and HPH10.0 were positioned on the positive side. CB was located near the center of the plot, indicating an intermediate response compared with the

control and HPH treatments. HPH7.5 and HPH10.0 were separated along PC2, suggesting different effects of the two HPH concentrations on yield and fruit quality traits. The loading plot showed that all studied traits were positively associated with PC1, indicating that this component represented an overall improvement in yield and fruit quality. HPH7.5 was closely associated with TY, NFP, NFrI, TFrN, FrL, FrW, TSS, and FrS. In contrast, HPH10.0 was more closely related to NFI, FrF, and FrWi. These results indicate that HPH7.5 was mainly associated with yield enhancement, fruit retention, average fruit fresh weight, and soluble solid accumulation, whereas HPH10.0 was more related to flower production, fruit firmness, and fruit width.

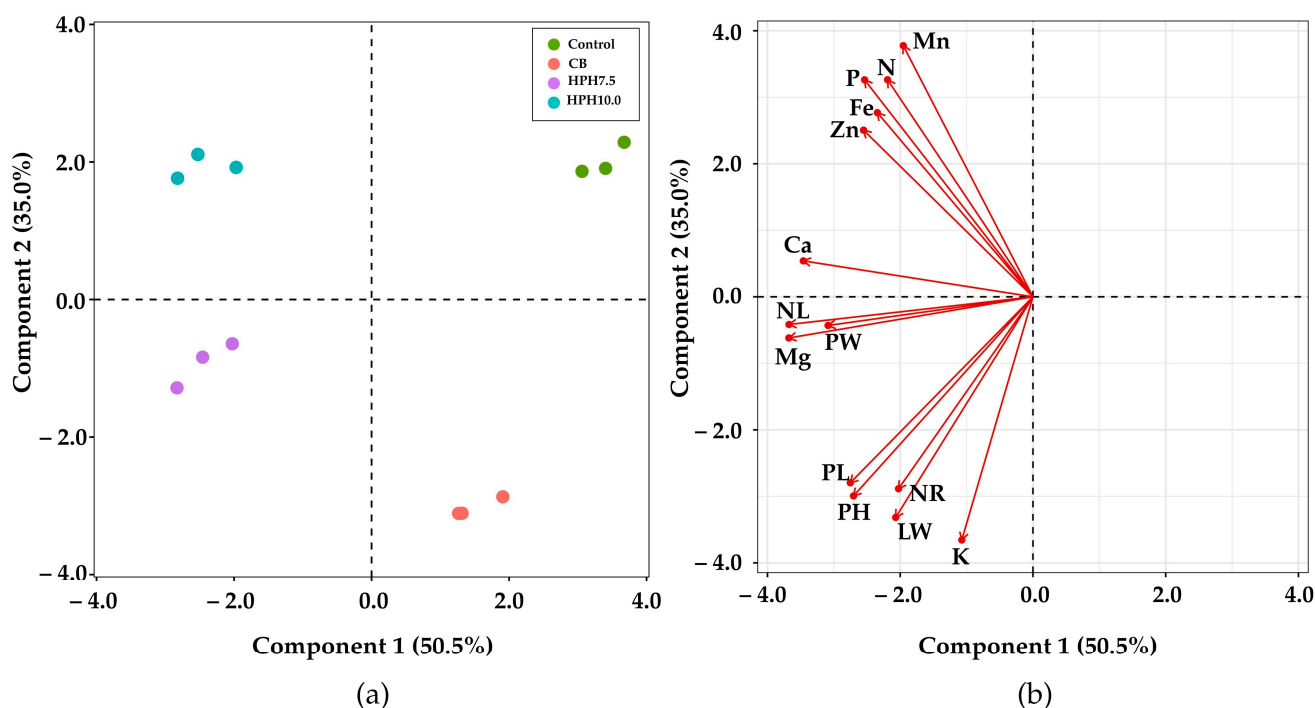


Figure 4. Principal component analysis of growth and mineral content responses in strawberry cv. 'Pharachatan 80' as affected by foliar HPH application under smart greenhouse conditions. (a) Score plot of treatments. (b) Loading plot of growth and mineral contents. LW: leaf width; NL: number of leaves plant⁻¹; NR: number of runners plant⁻¹; PH: plant height; PL: petiole length; PW: plant width.

For the bioactive compounds and antioxidant activity of strawberry fruits, PC1 and PC2 explained 81.5% and 13.5% of the total variance, respectively (Figure 6a,b). The score plot showed separation of treatments along PC1, with the control located on the negative side, CB near the center, and both HPH treatments on the positive side. HPH7.5 and HPH10.0 were separated along PC2, indicating different responses in antioxidant-related traits between the two HPH concentrations. The loading plot showed that all studied traits were positively associated with PC1, suggesting that this component represented an overall increase in bioactive compounds and antioxidant activity. HPH7.5 was closely related to TPC, TFC, TAsA, and ABTS, whereas HPH10.0 was more associated with TAC and DPPH. These results indicate that HPH7.5 mainly enhanced phenolic-, flavonoid-, ascorbic acid-, and ABTS-related antioxidant responses, while HPH10.0 was more closely related to anthocyanin accumulation and DPPH activity.

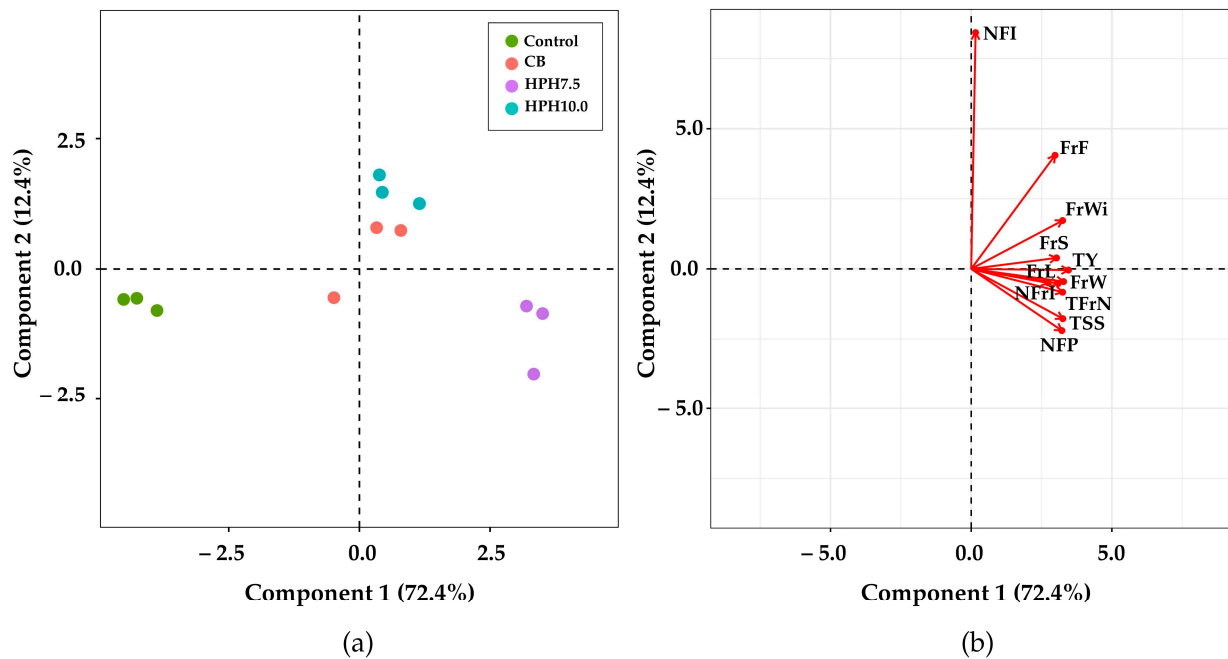


Figure 5. Principal component analysis of yield components and fruit quality responses in strawberry cv. 'Pharachatan 80' as affected by foliar HPH application under smart greenhouse conditions. (a) Score plot of treatments. (b) Loading plot of yield components and fruit quality. FrF: fruit firmness; NFI: number of flowers inflorescence⁻¹; FrL: fruit length; FrW: average fruit fresh weight; FrWi: fruit width; FrS: fruit set percentage; NFrI: number of fruits inflorescence⁻¹; NFP: number of inflorescences plant⁻¹; TFrN: total fruit number plant⁻¹; TSS: total soluble solids; TY: total yield.

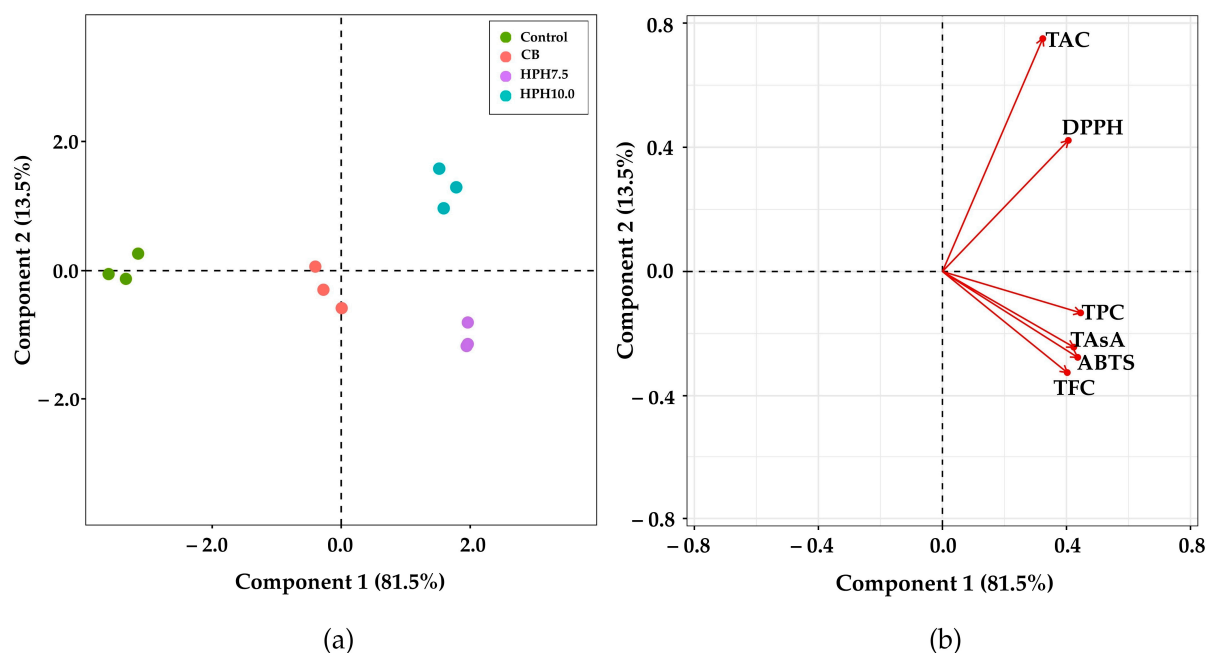


Figure 6. Principal component analysis of fruit bioactive compounds and antioxidant activity responses in strawberry cv. 'Pharachatan 80' as affected by foliar HPH application under smart greenhouse conditions. (a) Score plot of treatments. (b) Loading plot of fruit bioactive compounds and antioxidant activity. ABTS: ABTS antioxidant activity; DPPH: DPPH antioxidant activity; TAC: total anthocyanin content; TAsA: total ascorbic acid content; TFC: total flavonoid content; TPC: total phenolic content.

4. Discussion

Foliar application of the HPH formulation improved vegetative growth and mineral accumulation in strawberry cv. ‘Pharachatan 80’, although the responses differed between the two concentrations (Figure 3, Table 1). HPH7.5 was more closely associated with PH, PW, NL, PL, LW, runner production, and higher K and Mg contents, whereas HPH10.0 was more strongly related to the accumulation of N, P, Ca, Fe, Zn, and Mn. HPH7.5 increased mineral contents by 4.6–26.8% compared with the control, with the greatest increase observed for Mg, whereas HPH10.0 increased most mineral contents by 10.1–175.3%, except for K (Table 1). This pattern indicates a concentration-specific response, in which HPH7.5 favored vegetative development, while HPH10.0 promoted the accumulation of several mineral nutrients. The growth-promoting effect of HPH may be partly associated with free amino acids and short-chain peptides, which have been reported to function as readily available organic nitrogen sources and signaling compounds involved in carbon and nitrogen metabolism, enzyme activity, photosynthesis, and hormone-related regulation [15,16,41,42]. In addition, amino acids and peptides in PHs may improve nutrient mobility through chelating activity, thereby supporting nutrient uptake and translocation [30,43]. Similar responses were reported by Navarro-León et al. [17], in which L-amino acid-based biostimulants increased lettuce biomass, chlorophyll accumulation, photosynthetic performance, NO_3^- accumulation, total N accumulation, and nitrogen use efficiency. The present results are also consistent with our previous work, where hempseed press cake-derived PH improved vegetative growth and mineral contents in greenhouse-grown Red Oak lettuce [25]. The increased K and Mg contents under HPH7.5 may be consistent with a more favorable mineral status for photosynthesis and assimilate transport, while the higher Ca, Fe, and Zn contents under HPH10.0 may reflect enhanced mineral accumulation associated with the amino acid- and peptide-containing formulation. In strawberry, Ciriello et al. [13] reported that foliar application of vegetable-derived protein hydrolysate improved photosynthetic performance and enhanced leaf mineral profile, particularly NO_3^- , Ca^{2+} , and Mg^{2+} . Sopelsa et al. [20] also demonstrated that selected biostimulants, including alfalfa protein hydrolysate, promoted root biomass and leaf area development in strawberry plants grown under nutrient limitations. These findings support the interpretation that amino acid- and peptide-based biostimulants can improve vegetative growth, nutrient acquisition, and physiological performance in strawberry and other horticultural crops [21,27].

The improvement in vegetative growth under the HPH formulation was associated with reproductive development and yield formation under smart greenhouse conditions. The greenhouse system moderated external environmental stress by reducing the mean maximum temperature from 41.52 °C outside to 35.92 °C inside and lowering PAR from 1581.86 to 290.84 $\mu\text{mol m}^{-2} \text{s}^{-1}$. This reduction may be partly associated with the multi-functional greenhouse film, which provides high UV filtration and NIR reflection. Similar effects of greenhouse covering materials and thermal screen configuration on microclimate regulation and strawberry yield have been reported by Akpenpuun et al. [6]. In addition, tropical-adapted greenhouse systems have been shown to improve crop physiological performance and quality under warm conditions [7]. Nevertheless, the internal maximum temperature remained relatively high for strawberry reproductive development. Strawberry growth and fruit yield are strongly influenced by greenhouse microclimate, including temperature, light intensity, humidity, and daylength [9,10]. High temperature has also been reported to reduce inflorescence formation, flower number, fruit number, and fruit set in strawberries, thereby limiting yield potential [8,12]. Under this moderated but still suboptimal environment, HPH7.5 produced the highest NFP, NFrI, FrS, TFrN, TY, and FrW (Tables 2 and 3). Notably, HPH7.5 increased fruit set from 25.76% in control to 56.61% and increased TY, TFrN, and FrW by 161.7, 121.1, and 141.5%, respectively. In contrast, HPH10.0

increased NFI but did not result in the highest fruit set or yield, indicating that flower production alone was not sufficient to determine final productivity. Fruit set is a complex developmental process involving ovary growth, fruit initiation, and subsequent fruit development, and is regulated by hormonal interactions, particularly auxins, gibberellins, and cytokinins [44–46]. The superior fruit set under HPH7.5 suggested that this concentration may have supported plants to maintain a better balance between vegetative growth, reproductive development, and assimilate supply under the greenhouse environment.

PHs have been reported to exhibit hormone-like activity and to influence flowering, fruit set, and fruit development through their amino acid, oligopeptide, and polypeptide fractions [15,16]. Marfà et al. [19] also reported that animal-derived protein hydrolysate improved biomass accumulation, promoted flowering, and shortened the harvest period in strawberry, while amino acid-based biostimulants have been shown to enhance strawberry growth, yield, and resilience in soilless glasshouse cultivation [21]. Similarly, *Zea mays*-derived protein hydrolysate affected strawberry production, mineral profile, and fruit quality under tunnel cultivation, with responses depending on the application mode [23]. Such variation is also common in fruit crops treated with PH-based biostimulants, where plant responses may differ according to formulation type, crop species, and application strategy [22]. In the present study, HPH7.5 increased both fruit number and fruit size, suggesting that this treatment may have supported fruit development without an apparent trade-off between fruit number and fruit weight. The balance between fruit number and fruit size is important because excessive fruit load can reduce fruit size when assimilate supply is limited [47]. Thus, the beneficial effect of HPH7.5 on fruit set and yield was particularly relevant under the present smart greenhouse conditions, where environmental control reduced external stress but did not fully provide an optimal reproductive environment for strawberries.

HPH application also improved fruit quality attributes of strawberry, although the response differed between concentrations. HPH7.5 produced the highest FrL and TSS, whereas HPH10.0 resulted in the highest FrF (Table 3). Compared with the control, HPH7.5 increased FrL, FrWi, FrF, and TSS by 56.2, 34.2, 75.9, and 95.5%, respectively, while HPH10.0 produced the greatest increase in FrF. The increase in TSS under HPH7.5 may be associated with improved assimilate availability and translocation of soluble carbohydrates to developing fruits. TSS is an important quality indicator in strawberry because it is closely associated with soluble sugars, organic acids, amino acids, vitamins, and other compounds that contribute to sweetness, flavor perception, and consumer acceptance [48–50]. PHs, particularly those containing free amino acids and small peptides, have been reported to stimulate photosynthetic activity, chlorophyll biosynthesis, and carbon metabolism, which could contribute to sugar accumulation in sink organs such as fruits [51,52]. In the present study, the higher TSS and larger fruit size under HPH7.5 were consistent with their positive effects on vegetative growth, FrS, and TY, suggesting improved assimilate supply to developing fruits. In contrast, the greater firmness observed under HPH10.0 may be partly linked to improved plant Ca status, as calcium contributes to cell wall stability, membrane integrity, and delayed fruit softening [53]. Plant-derived biostimulants and PHs may also influence cell wall-related processes, including the synthesis and modification of pectin, cellulose, and hemicellulose, as well as enzymes involved in cell wall degradation during ripening [54]. These results indicate that HPH7.5 was more effective in improving sweetness-related quality and fruit size, whereas HPH10.0 was more closely related to firmness, suggesting concentration-specific effects of HPH on different aspects of strawberry fruit quality.

HPH application also enhanced fruit bioactive compounds and antioxidant activity, indicating that the HPH formulation may be associated with changes in antioxidant-

related fruit metabolism. HPH7.5 was more effective in increasing TPC, TFC, TAsA, and ABTS activity, whereas HPH10.0 produced the highest TAC and DPPH activity (Table 4). Compared with the control, HPH7.5 increased TPC, TFC, TAsA, and ABTS antioxidant activity by 64.8, 81.8, 128.2, and 197.3%, respectively, whereas HPH10.0 increased TAC and DPPH antioxidant activity by 56.2 and 91.0%, respectively. This concentration-specific response suggests that different HPH levels may differentially affect antioxidant-related metabolism in strawberry fruits. The increase in TPC and TFC under HPH7.5 may be associated with the amino acid and peptide composition of PHs, particularly phenylalanine, which serves as a precursor for the phenylpropanoid pathway. This pathway is initiated by phenylalanine ammonia-lyase and leads to the biosynthesis of phenolic acids, flavonoids, lignin, tannins, and anthocyanins, which contribute to antioxidant capacity in fruits [30,51]. The higher TAsA and ABTS activity under HPH7.5 suggest improved antioxidant-related fruit quality, while the greater TAC and DPPH activity under HPH10.0 may reflect a stronger stimulation of anthocyanin-associated antioxidant responses. Similar responses have been reported in strawberry, where biostimulant application enhanced phenolic compounds, flavonoids, anthocyanins, and antioxidant potential, although the magnitude and direction of responses depended on biostimulant type, crop condition, and fruit metabolic status [13,26–28].

PCA explained 85.5, 84.8, and 95.0% of the total variance for growth–mineral traits, yield–quality traits, and bioactive–antioxidant traits, respectively (Figures 4–6). The PCA results provided an integrated view of these responses and supported the interpretation that HPH7.5 and HPH10.0 were associated with distinct response patterns. In the PCA of growth and mineral traits, HPH7.5 was more closely associated with vegetative growth traits, K, and Mg, whereas HPH10.0 was associated with N, P, Ca, Fe, Zn, and Mn (Figure 4). In the PCA of yield and fruit quality, HPH7.5 was grouped with TY, TFrN, FrS, TSS, and FrL, while HPH10.0 was more closely related to NFI, FrF, and FrWi (Figure 5). Similarly, in the PCA of bioactive compounds and antioxidant activity, HPH7.5 was associated with TPC, TFC, TAsA, and ABTS antioxidant activity, whereas HPH10.0 was associated with TAC and DPPH antioxidant activity (Figure 6). This multivariate separation suggests that HPH7.5 was associated with a more balanced improvement across growth, yield, fruit quality, and antioxidant-related traits, while HPH10.0 enhanced more specific traits related to mineral accumulation, firmness, anthocyanin accumulation, and DPPH antioxidant activity.

The improvements in growth, yield components, fruit quality, and antioxidant-related traits may be linked to photosynthetic performance, hormone-like signaling, source–sink relationships, and phenylpropanoid metabolism. Because these processes were not measured directly, the proposed links should be interpreted with caution. Future studies should also examine leaf-level biochemical and physiological responses, as the HPH formulation was applied directly to the foliage. Relevant measurements may include antioxidant status, photosynthetic pigments and parameters, hormone profiling, and targeted metabolite analysis. The results should also be interpreted at the formulation level, since HPH was applied as part of a complete foliar formulation rather than as HPH alone. Information on peptide profiles, degree of hydrolysis, and mineral composition would help refine the formulation. Multi-season and multi-cultivar trials are still needed to confirm its practical use in strawberry production.

5. Conclusions

Foliar application of HPH improved the growth, mineral accumulation, yield, fruit quality, and antioxidant-related traits of strawberry cv. ‘Pharachatan 80’ under smart greenhouse conditions. Among the treatments, HPH7.5 was the most effective, enhancing vegetative growth, K and Mg accumulation, fruit set, fruit number, total yield, fruit size, TSS,

TPC, TFC, TAsA, and ABTS antioxidant activity. Conversely, HPH10.0 was more closely associated with the accumulation of selected minerals, FrF, TAC, and DPPH antioxidant activity, indicating a concentration-specific response. PCA supported the interpretation that HPH7.5 provided a more balanced improvement across agronomic and fruit quality traits. Therefore, HPH at 7.5% (*w/v*) appears to be an effective foliar biostimulant for improving strawberry productivity and fruit quality under smart greenhouse production.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae12080958/s1>, Figure S1: Environmental conditions recorded inside and outside the smart greenhouse during the experimental period from January to March 2026. (A) Minimum and maximum air temperature (°C); (B) minimum and maximum relative humidity (%); and (C) photosynthetically active radiation (PAR; $\mu\text{mol m}^{-2} \text{s}^{-1}$); Table S1: Amino acid composition ($\text{mg } 100 \text{ g}^{-1} \text{ DW}$) of hempseed press cake-derived protein hydrolysate (HPH) and the commercial biostimulant (CB) used in this study; Table S2: pH and electrical conductivity (EC) of the foliar spraying solutions used in this study.

Author Contributions: Conceptualization, B.H.; methodology, B.H.; software, L.C. and B.H.; validation, O.T. and B.H.; formal analysis, T.T. and B.H.; investigation, T.T., L.C. and B.H.; resources, B.H.; data curation, B.H.; writing—original draft preparation, B.H.; writing—review and editing, O.T. and B.H.; visualization, B.H.; supervision, B.H.; project administration, B.H.; funding acquisition, B.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Thailand Science Research and Innovation (TSRI) Fundamental Fund, fiscal year 2026, Thammasat University (Grant no. TUFF 21/2569).

Data Availability Statement: The original contributions presented in this study are included in the article and Supplementary Materials. Further inquiries can be directed to the corresponding author.

Acknowledgments: The authors gratefully acknowledge the Department of Agricultural Technology, Faculty of Science and Technology, Thammasat University, Pathum Thani, Thailand, for providing research facilities and infrastructure support.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

AAS	atomic absorption spectrophotometry
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay
ANOVA	analysis of variance
CB	commercial biostimulant
CEA	controlled-environment agriculture
CRD	completely randomized design
DAT	days after transplanting
DPPH	2,2-diphenyl-1-picrylhydrazyl radical scavenging assay
DW	dry weight
FrF	fruit firmness
FrL	fruit length
FrW	average fruit fresh weight
FrWi	fruit width
FrS	fruit set percentage
FW	fresh weight
HPH	hempseed press cake-derived protein hydrolysate
NFP	number of inflorescences plant^{-1}
NFI	number of flowers inflorescence $^{-1}$

NFrI	number of fruits inflorescence ^{−1}
NIR	near-infrared
NL	number of leaves plant ^{−1}
NR	number of runners plant ^{−1}
PAR	photosynthetically active radiation
PCA	principal component analysis
PH	plant height
PHs	protein hydrolysates
PL	petiole length
PW	plant width
TAC	total anthocyanin content
TAsA	total ascorbic acid content
TFC	total flavonoid content
TFrN	total fruit number plant ^{−1}
TPC	total phenolic content
TSS	total soluble solids
Tukey's HSD	Tukey's honestly significant difference
TY	total yield

References

1. Yang, J.-W.; Kim, H.-I. An overview of recent advances in greenhouse strawberry cultivation using deep learning techniques: A review for strawberry practitioners. *Agronomy* **2024**, *14*, 34. [\[CrossRef\]](#)
2. Choi, J.Y.; Seo, K.; Cho, J.S.; Moon, K.D. Applying convolutional neural networks to assess the external quality of strawberries. *J. Food Compos. Anal.* **2021**, *102*, 104071. [\[CrossRef\]](#)
3. Wang, F.; Wang, J.; Ji, G.; Kang, X.; Li, Y.; Hu, J.; Qian, C.; Wang, S. Regulation of anthocyanins and quality in strawberries based on light quality. *Horticulturae* **2025**, *11*, 377. [\[CrossRef\]](#)
4. Klein, A.P.; Mulinari, J.; Reichert Junior, F.W.; Trentin, T.d.S.; Aguilar, M.G.d.; Machado, A.R.T.; Oliveira, D.F.d.; Colla, L.M.; Chiomento, J.L.T. Potential of strawberry leaves with biostimulants: Repository of metabolites and bioethanol production. *Processes* **2025**, *13*, 3244. [\[CrossRef\]](#)
5. Dal Magro, S.Z.; Chiomento, J.L.T.; Werner, H.A.; Bortoluzzi, E.C.; Bortoluzzi, M.P. Enhancing greenhouse strawberry irrigation: Integrating IoT technologies and low-cost moisture sensors in substrate. *Rev. Cad. Pedagógico* **2024**, *21*, e7258. [\[CrossRef\]](#)
6. Akpenpuun, T.D.; Na, W.-H.; Ogunlowo, Q.O.; Rabiou, A.; Adesanya, M.A.; Addae, K.S.; Kim, H.-T.; Lee, H.-W. Effect of greenhouse cladding materials and thermal screen configuration on heating energy and strawberry (*Fragaria ananassa* var. "Seolhyang") yield in winter. *Agronomy* **2021**, *11*, 2498. [\[CrossRef\]](#)
7. Kumsong, N.; Thepsilvisut, O.; Imorachorn, P.; Chutimanukul, P.; Pimpha, N.; Toojinda, T.; Trithaveesak, O.; Ratanaudomphisut, E.; Poyai, A.; Hruanun, C.; et al. Comparison of different temperature control systems in tropical-adapted greenhouses for green romaine lettuce production. *Horticulturae* **2023**, *9*, 1255. [\[CrossRef\]](#)
8. Ledesma, N.A.; Sugiyama, N. Effect of high temperature stress on the reproductive growth of strawberry cvs. 'Nyoho' and 'Toyonoka'. *Sci. Hortic.* **2008**, *116*, 186–193. [\[CrossRef\]](#)
9. Sim, H.S.; Kim, D.S.; Ahn, M.G.; Ahn, S.R.; Kim, S.K. Prediction of strawberry growth and fruit yield based on environmental and growth data in a greenhouse for soil cultivation with applied autonomous facilities. *Hortic. Sci. Technol.* **2020**, *38*, 840–849. [\[CrossRef\]](#)
10. Kim, S.; Jo, J.S.; Luk, V.; Kim, S.K.; Kim, D.S. Estimating the impact of environmental management on strawberry yield using publicly available agricultural data in South Korea. *PeerJ.* **2023**, *11*, e15390. [\[CrossRef\]](#) [\[PubMed\]](#)
11. Naito, H.; Kawasaki, Y.; Hidaka, K.; Higashide, T.; Misumi, M.; Ota, T.; Lee, U.; Takahashi, M.; Hosoi, F.; Nakagawa, J. Effect of air temperature on each fruit growth and ripening stage of strawberry 'Koiminori'. *Int. Agrophys.* **2024**, *38*, 195–202. [\[CrossRef\]](#)
12. Nishiyama, M.; Kanayama, Y. Effects of high temperature on the fruit development and sugar concentration in everbearing strawberry cultivars. *Adv. Hortic. Sci.* **2025**, *39*, 261–268. [\[CrossRef\]](#)
13. Ciriello, M.; Pannico, A.; Roupael, Y.; Basile, B. Enhancing yield, physiological, and quality traits of strawberry cultivated under organic management by applying different non-microbial biostimulants. *Plants* **2025**, *14*, 712. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Štyriaková, D.; Hajnal-Jafari, T.; Žunić, V.; Šuba, J.; Prekopová, M.; Yetik, A.K.; Štyriaková, I. Influence of biostimulant applications on vegetative growth and yield of strawberry under full and reduced fertilization. *DYSONA-Appl. Sci.* **2026**, *7*, 41–49. [\[CrossRef\]](#)

15. Colla, G.; Rouphael, Y.; Lucini, L.; Canaguier, R.; Stefanoni, W.; Fiorillo, A.; Cardarelli, M. Protein hydrolysate-based biostimulants: Origin, biological activity and application methods. In Proceedings of the II World Congress on the Use of Biostimulants in Agriculture, Florence, Italy, 16–19 November 2015; p. 1148. [\[CrossRef\]](#)
16. Colla, G.; Hoagland, L.; Ruzzi, M.; Cardarelli, M.; Bonini, P.; Canaguier, R.; Rouphael, Y. Biostimulant action of protein hydrolysates: Unraveling their effects on plant physiology and microbiome. *Front. Plant Sci.* **2017**, *8*, 2202. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Navarro-León, E.; López-Moreno, F.J.; Borda, E.; Marín, C.; Sierras, N.; Blasco, B.; Ruiz, J.M. Effect of L-amino acid-based biostimulants on nitrogen use efficiency (NUE) in lettuce plants. *J. Sci. Food Agric.* **2022**, *102*, 7098–7106. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Sun, W.; Shahrajabian, M.H.; Kuang, Y.; Wang, N. Amino acids biostimulants and protein hydrolysates in agricultural sciences. *Plants* **2024**, *13*, 210. [\[CrossRef\]](#) [\[PubMed\]](#)
19. Marfà, O.; Cáceres, R.; Polo, J.; Ródenas, J. Animal protein hydrolysate as a biostimulant for transplanted strawberry plants subjected to cold stress. *Acta Hort.* **2009**, *842*, 315–318. [\[CrossRef\]](#)
20. Soppelsa, S.; Kelderer, M.; Casera, C.; Bassi, M.; Robatscher, P.; Matteazzi, A.; Andreotti, C. Foliar applications of biostimulants promote growth, yield and fruit quality of strawberry plants grown under nutrient limitation. *Agronomy* **2019**, *9*, 483. [\[CrossRef\]](#)
21. Ranasingha, R.; Perera, A.; Baghalian, K.; Gerofotis, C. Amino acid-based biostimulants and microbial biostimulants promote the growth, yield and resilience of strawberries in soilless glasshouse cultivation. *J. Sustain. Agric. Environ.* **2024**, *3*, e12113. [\[CrossRef\]](#)
22. Dlačić, I.; Chatzistathis, T.; Bernobić, S.; Polić Pasković, M.; Ban, D.; Petek, M.; Popović, L.; Pasković, I. Protein hydrolyzate-based biostimulants in fruit crops: Mechanisms, agronomic effects, and practical applications. *Crops* **2026**, *6*, 30. [\[CrossRef\]](#)
23. Mancuso, F.; Vultaggio, L.; Sabatino, L.; Bellitto, P.; Ntatsi, G.; Allevato, E.; La Placa, G.G.; La Bella, S.; Consentino, B.B. *Zea mays*-derived protein hydrolysate and diverse application modes differentially compose crop production and fruit quality of strawberry cultivated under tunnel. *Agronomy* **2025**, *15*, 1314. [\[CrossRef\]](#)
24. Pasković, I.; Andlovic, M.; Plešnik, H.; Vavpetič, P.; Žurga, P.; Popović, L.; Šala, M.; Franić, M.; Dlačić, I.; Goreta Ban, S.; et al. Foliar application of protein hydrolysates promotes growth and affects leaf ionome in olive. *Horticulturae* **2026**, *12*, 151. [\[CrossRef\]](#)
25. Harakotr, B.; Trisiri, T.; Charoensup, L.; Thepsilvisut, O.; Rithichai, P.; Suwor, P.; Jirakiattikul, Y. Effects of protein hydrolysate derived from hempseed by-products on growth, mineral contents, and quality of greenhouse grown red oak lettuce. *Horticulturae* **2025**, *11*, 357. [\[CrossRef\]](#)
26. Garza-Alonso, C.A.; Olivares-Sáenz, E.; González-Morales, S.; Cabrera-De la Fuente, M.; Juárez-Maldonado, A.; González-Fuentes, J.A.; Tortella, G.; Valdés-Caballero, M.V.; Benavides-Mendoza, A. Strawberry biostimulation: From mechanisms of action to plant growth and fruit quality. *Plants* **2022**, *11*, 3463. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Soltaniband, V.; Brégard, A.; Gaudreau, L.; Dorais, M. Biostimulants promote plant development, crop productivity, and fruit quality of protected strawberries. *Agronomy* **2022**, *12*, 1684. [\[CrossRef\]](#)
28. Vultaggio, L.; Allevato, E.; Consentino, B.B.; Bellitto, P.; Napoli, S.; Cannata, C.; Ntatsi, G.; Vasto, S.; Baldassano, S.; La Bella, S.; et al. Joint action of *Trichoderma atroviride* and a vegetal derived-protein hydrolysate improves performances of woodland strawberry in Italy. *Horticulturae* **2024**, *10*, 459. [\[CrossRef\]](#)
29. Ertani, A.; Cavani, L.; Pizzeghello, D.; Brandellero, E.; Altissimo, A.; Ciavatta, C.; Nardi, S. Biostimulant activity of two protein hydrolyzates in the growth and nitrogen metabolism of maize seedlings. *J. Plant Nutr. Soil Sci.* **2009**, *172*, 237–244. [\[CrossRef\]](#)
30. Cardarelli, M.; El Chami, A.; Rouphael, Y.; Ciriello, M.; Bonini, P.; Erice, G.; Cirino, V.; Basile, B.; Corrado, G.; Choi, S.; et al. Plant Biostimulants as natural alternatives to synthetic auxins in strawberry production: Physiological and metabolic insights. *Front. Plant Sci.* **2024**, *14*, 1337926. [\[CrossRef\]](#) [\[PubMed\]](#)
31. Capcanari, T.; Covaliov, E.; Negoita, C.; Siminiuc, R.; Chirsanova, A.; Reșitca, V.; Țurcanu, D. Hemp seed cake flour as a source of proteins, minerals and polyphenols and its impact on the nutritional, sensorial and technological quality of bread. *Foods* **2023**, *12*, 4327. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Danilov, I.; Vlajkov, V.; Šumić, Z.; Milić, A.; Horecki, A.T.; Dujković, T.; Živanović, N.; Simin, N.; Lesjak, M.; Grahovac, J. Valorization of strawberry juice production wastewater: Possibilities for polyphenols recovery and plant biostimulant production. *Foods* **2024**, *13*, 3224. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Nazeer, S.; Agosti, A.; Leto, L.; Amini, A.; Remelli, A.; Fazio, A.D.; Turolla, A.; Paterna, A.; Rao, E.; Cirlini, M.; et al. Effect of circular-economy-derived experimental biostimulants on growth and development of soilless strawberry plants in greenhouse conditions. *J. Sustain. Agric. Environ.* **2025**, *4*, e70101. [\[CrossRef\]](#)
34. Jirakiattikul, Y.; Ruangnoo, S.; Sangmukdee, K.; Chamchusri, K.; Rithichai, P. Enhancement of plumbagin production through elicitation in in vitro-regenerated shoots of *Plumbago indica* L. *Plants* **2024**, *13*, 1450. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Folin, O.; Ciocalteu, V. On tyrosine and tryptophane determinations in proteins. *J. Biol. Chem.* **1927**, *73*, 627–650. [\[CrossRef\]](#)
36. Kubola, J.; Siriamornpun, S.; Meeso, N. Phytochemicals, vitamin C and sugar content of Thai wild fruits. *Food Chem.* **2011**, *126*, 972–981. [\[CrossRef\]](#)
37. Lee, J.; Durst, R.W.; Wrolstad, R.E. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. *J. Aoac Int.* **2005**, *88*, 1269–1278. [\[CrossRef\]](#)

38. Bassi, M.; Lubes, G.; Bianchi, F.; Agnolet, S.; Ciesa, F.; Brunner, K.; Guerra, W.; Robatscher, P.; Oberhuber, M. Ascorbic acid content in apple pulp, peel, and monovarietal cloudy juices of 64 different cultivars. *Int. J. Food Prop.* **2018**, *20*, S2626–S2634. [[CrossRef](#)]
39. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *Leb. Wiss Technol.* **1995**, *28*, 25–30. [[CrossRef](#)]
40. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [[CrossRef](#)] [[PubMed](#)]
41. Sarmadi, B.H.; Ismail, A. Antioxidative peptides from food proteins: A review. *Peptides* **2010**, *31*, 1949–1956. [[CrossRef](#)] [[PubMed](#)]
42. Alfosea-Simón, M.; Simón-Grao, S.; Zavala-Gonzalez, E.A.; Cámara-Zapata, J.M.; Simón, I.; Martínez-Nicolás, J.J.; Lidón, V.; Rodríguez-Ortega, W.M.; García-Sánchez, F. Application of biostimulants containing amino acids to tomatoes could favor sustainable cultivation: Implications for tyrosine, lysine, and methionine. *Sustainability* **2020**, *12*, 9729. [[CrossRef](#)]
43. Colla, G.; Rouphael, Y.; Canaguier, R.; Svecova, E.; Cardarelli, M. Biostimulant action of a plant-derived protein hydrolyzates produced through enzymatic hydrolysis. *Front. Plant Sci.* **2014**, *5*, 448. [[CrossRef](#)] [[PubMed](#)]
44. Gillasp, G.E.; Ben-David, H.; Gruissem, W. Fruits: A developmental perspective. *Plant Cell* **1993**, *5*, 1439–1451. [[CrossRef](#)] [[PubMed](#)]
45. Kumar, R.; Khurana, A.; Sharma, A.K. Role of plant hormones and their interplay in development and ripening of fleshy fruits. *J. Exp. Bot.* **2014**, *65*, 4561–4575. [[CrossRef](#)] [[PubMed](#)]
46. Fenn, M.A.; Giovannoni, J.J. Phytohormones in fruit development and maturation. *Plant J.* **2020**, *105*, 446–458. [[CrossRef](#)] [[PubMed](#)]
47. Whiting, M.D.; Lang, G.A. ‘Bing’ sweet cherry on the dwarfing rootstock ‘Gisela 5’: Thinning affects fruit quality and vegetative growth but not net CO₂ exchange. *J. Amer. Soc. Hort. Sci.* **2004**, *129*, 407–415. [[CrossRef](#)]
48. Krüger, E.; Josuttis, M.; Nestby, R.; Toldam-Andersen, T.B.; Carlen, C.; Mezzetti, B. Influence of growing conditions at different latitudes of Europe on strawberry growth performance, yield and quality. *J. Berry Res.* **2012**, *2*, 143–157. [[CrossRef](#)]
49. Urün, I.; Attar, S.H.; Sönmez, D.A.; Gündeşli, M.A.; Ercişli, S.; Kafkas, N.E.; Bandić, L.M.; Duralija, B. Comparison of polyphenol, sugar, organic acid, volatile compounds, and antioxidant capacity of commercially grown strawberry cultivars in Turkey. *Plants* **2021**, *10*, 1654. [[CrossRef](#)] [[PubMed](#)]
50. Milosavljević, D.; Maksimović, V.; Miliivojević, J.; Djekić, I.; Wolf, B.; Zuber, J.; Vogt, C.; Dragišić Maksimović, J. Sugars and organic acids in 25 strawberry cultivars: Qualitative and quantitative evaluation. *Plants* **2023**, *12*, 2238. [[CrossRef](#)] [[PubMed](#)]
51. Ertani, A.; Schiavon, M.; Nardi, S. Transcriptome-wide identification of differentially expressed genes in *Solanum lycopersicon* L. in response to an *alfalfa*-protein hydrolysate using microarrays. *Front. Plant Sci.* **2017**, *8*, 1159. [[CrossRef](#)] [[PubMed](#)]
52. García-Cano, C.; Ferrández-Gómez, B.; Jordá, J.D.; Cerdán, M.; Sánchez-Sánchez, A. Comparative evaluation of organic and conventional biostimulant application in strawberry (*Fragaria × ananassa*) plants: Effects on flowering, ripening, and fruit quality. *J. Agric. Food Res.* **2025**, *23*, 102180. [[CrossRef](#)]
53. Hocking, B.; Tyerman, S.D.; Burton, R.A.; Gilliam, M. Fruit calcium: Transport and physiology. *Front. Plant Sci.* **2016**, *7*, 569. [[CrossRef](#)] [[PubMed](#)]
54. Basile, B.; Brown, N.; Valdes, J.M.; Cardarelli, M.; Scognamiglio, P.; Mataffo, A.; Rouphael, Y.; Bonini, P.; Colla, G. Plant-based biostimulant as sustainable alternative to synthetic growth regulators in two sweet cherry cultivars. *Plants* **2021**, *10*, 619. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.