ORIGINAL RESEARCH ARTICLE

Enhancing strawberry fruit growth in hydroponic greenhouse: Synergistic effects of biochar and humic acid

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ABSTRACT

Hydroponics is a modern agricultural system that enables year-round plant growth. Biochar, derived from apple tree waste, and humic acid were investigated as a replacement for the Hoagland nutrient solution to grow strawberries in a greenhouse with three replications. Growth parameters, such as leaf area, the average number of fruits per plant, maximum fruit weight, and the weight of fresh and dry fruits, were measured. A 50% increase in fresh and dry fruit weight was observed in plants grown using biochar compared to the control. Additionally, the use of Hoagland chemical fertilizer led to a 25% increase in both fresh and dry weight. There was a 65% increase in the number of fruits per plant in the biochar-grown sample compared to the control. Moreover, biochar fertilizer caused a 100% increase in maximum fruit weight compared to the control and a 27% increase compared to the Hoagland chemical fertilizer. Biochar had a higher pH compared to the Hoagland solution, and such pH levels were conducive to strawberry plant growth. The results indicate that biochar has the potential to enhance the size and weight of fruits. The findings of the study demonstrate that biochar, when combined with humic acid, is a successful organic hydroponic fertilizer that improves the quality and quantity of strawberries. Moreover, this approach enables the more efficient utilization of garden waste.

Keywords: hydroponic; biochar; strawberry; humic acid; Parus variety

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1. Introduction

The availability of fresh and high-quality strawberries throughout the year is an advantage of hydroponic greenhouse farming. Agriculture currently has harmful impacts on the environment. According to FAO^[1], agriculture is responsible for over 70% of water consumption worldwide. These impacts are mostly attributed to poorly functioning irrigation systems or damages incurred by such systems. Additionally, indiscriminate consumption practices on farms contribute to these negative effects. Hydroponic greenhouse farming is a plant cultivation method that uses an inert substrate layer of cocopeat and perlite and delivers organic matter and minerals to plant roots via a water-based nutrient solution instead of soil^[2]. Hydroponic gardening allows for better pest control compared to soil gardening. This system significantly reduces the expense of pesticides while also producing higher quality and healthier products^[3]. Open growth cultivation is one of the techniques used in hydroponic horticulture. In this method, water and mineral circulation is not done in a closed system and exits the system after passing through the plants. This is one of the most commonly used systems in Mediterranean greenhouses^[4]. Changing climate is a complex and ongoing phenomenon that alters climatic conditions, impacting both biotic and abiotic aspects. This causes alterations in weather patterns like extreme heat, rainfall, and temperatures. In turn, this leads to the emergence of novel parasites, vegetation, and diseases, while agricultural crops must adopt specific methods such as physiological, biochemical, and molecular processes to ensure optimal development during stressful situations^[5].

The strawberry (*Fragaria Ananassa* Duch) is a desirable fruit due to its distinct characteristics, such as its bright red color, fragrant aroma, soft texture, and slightly acidic flavor. This coloration is caused by the presence of bioactive substances, namely anthocyanins and flavonoids, which are potent antioxidants and exhibit anti-carcinogenic properties^[6].

The use of chemical fertilizers produced through inorganic formulations for different plant varieties can cause an imbalance in natural ecosystems^[7]. Fertilizers resulting from agricultural systems can have a low capability for water leakage into underground waters, which minimizes their negative effects on the environment. For instance, they create pollution in underground waterholes and lead to acidification and changes in the microbial diversity of soil and natural ecosystems. However, open hydroponic systems are also vulnerable to such hazards^[8]. The long-term use of chemical fertilizers causes the accumulation of nitrates in plants and underground water, leading to toxicity in both plants and humans^[9]. Biochar, also known as biocoal, is a solid substance composed of plant biomasses and agricultural wastes. It is a solid substance with a high carbon content that can remain in the soil for thousands of years. Biochar consists of varying amounts of carbon, hydrogen, oxygen, nitrogen, sulfur, and ash. Biochar production is an effective waste management strategy. The production of agricultural waste leads to more efficient and innovative methods for managing it^[10]. Agricultural waste generally contains glycocellulosic materials such as hemicellulose and lignin. Hemicellulose is composed of long, highly branched chains of sugars. Lignin is composed of monomers linked together in the form of molecules with long, branched chains. The compositions of hemicellulose and lignin vary between plant species. Biochar is a high-carbon by-product that is produced by heating biomass under oxygen-free or low-oxygen conditions^[11,12].

Biochar has gained significant traction as a valuable resource in Asian and tropical agricultural practices^[13]. The effects of biochar on plant species vary based on temperature fluctuations, leading to variations in the amount of biochar applied and its impact on plants^[14]. Biomass samples release more nitrogen during the pyrolysis process, resulting in a lower amount of residual nitrogen in biochar ash^[15]. Biochar ash, obtained from pruned plant waste, can be an eco-friendly and cost-effective source of plant nutrition. With its abundance of essential nutrients for plants, this natural resource serves as a nutritious and eco-conscious substitute for synthetic fertilizers^[16]. Some studies suggest that modifying biochar with organic compounds can increase root nutrient absorption and enhance plant growth^[17]. Humic acid compounds, characterized by their molecular complexes of organic compounds, possess notable characteristics such as being large in size, dark in color, and imbued with an aromatic essence. The exchange capacity and ability of humic acid compounds to form complex metal ions and aqueous oxides impact nutrient availability for plant roots and biological systems. Humic acid can be extracted from a diverse range of natural sources, including peat, compost, soil, sediments, and Leonardite coal, with extraction rates typically ranging from 45% to 85%, contingent upon prevailing conditions^[18,19]. Humic acid is primarily used in the agricultural industry^[20]. Studies have shown that humic acid is beneficial for phosphate absorption, and some reports suggest that it can enhance the root's adsorption of more nitrogen and other nutrients. Organic fertilizers are typically insoluble in water, resulting in slower nutrient availability. Liquid fertilizers can be applied to hydroponic plants. In a previous study, it was found that the performance of plants grown with biochar ash as an organic fertilizer is comparable to that of chemical fertilizers obtained from minerals^[11].

Humic acids (HA) are organic compounds that serve important functions in enhancing soil properties, plant growth, and agronomic and horticultural aspects. The sources of HA encompass coal, lignite, soils, and

organic matter. Humic acid-derived products have recently been implemented in crop production to promote sustainable agriculture practices^[21]. Furthermore, according to recent studies^[22,23], humic acids have the ability to enhance water retention and promote microbial growth while also increasing the availability of micronutrients to plants through chelation and co-transportation processes. They also play a crucial role in decreasing toxic heavy metal transportation by precipitating them, thus reducing plant uptake of these harmful elements^[24].

The objective of this study was to assess the efficacy of utilizing apple tree plant waste biochar in conjunction with humic acid as a liquid fertilizer, as compared to Hoagland's nutrient solution, in an open greenhouse hydroponic cultivation system for strawberry plants.

2. Materials and methods

In the research greenhouse of the Biology Department at Shiraz University, experiments were conducted to evaluate and compare the strawberry yield between two treatments: Biochar bioliquid fertilizer supplemented with humic acid and Hoagland's nutrient solution. The Parus variety of strawberry (*F. ananassa* Duch. cv. Parus) was used in the experiments. The temperature inside the greenhouse ranged between 21 °C and 26 °C during the day and between 16 °C and 19 °C at night. The relative humidity inside the greenhouse ranged between 55% and 65%. The average light intensity of the net-covered greenhouse on hot days was measured to be 15,000 lux using a flash meter and a light meter (Seconic-L758DR-U).

For the preparation of biochar, apple tree green waste, comprising branches and leaves that are typically discarded at the end of the production season, was utilized. The apple orchard chosen for this purpose was located in the Qalat area at coordinates 29.83° N and 52.33° E. The samples were collected randomly from ten areas of the garden's top, middle, and bottom plant sections using pruning shears. Once collected, the pruned samples were chopped and completely crushed using a mill. The sample was homogenized and then placed in the oven for 4 h. Subsequently, the dry weight was measured. All samples were meticulously weighed with a precision of 0.01 g. Thermal decomposition was carried out in a furnace set at a temperature of 500 °C, resulting in the production of biochar. The selected temperature of 500 °C was determined to be optimal for producing acceptable levels of ash, minerals, and electric conductivity. After preparation, the sample was poured into a crucible and kept in an electric furnace (model AFE1400L-45DH) for six hours. Subsequently, once cooled down, the sample was weighed and subsequently passed through a 2 mm sieve to ensure homogenization. To minimize testing errors, consistent production principles were applied across consecutive cultivation periods for fertilizer production. The production of the liquid fertilizer entailed the utilization of a liquid slurry comprising 5.5 g/L of biochar and 3.5 g/L of humic acid. The mixing of biochar ash and humic acid was conducted individually in a glass beaker using a magnetic stirrer (Alpha model HS860) for a period of 12 h at a temperature of 50 °C. The pH, electrical conductivity (EC), and total dissolved solids (TDS) were measured during the experiments using a Hach multimeter (model HQ30D). The volume of the liquid was increased, and pH adjustment was performed by adding 68% HNO3 to a total volume of 50 L of the liquid mixture.

To produce the fertilizer following the Hoagland method specified in **Table 1**^[25], all elements, except for sequestrene iron, were combined in a glass beaker using a magnetic stirrer for a duration of 6 h at a temperature of 50 °C. Once the volume of the solution was increased, sequestrene iron was added to the mixture.

2.1. Cultivation conditions

The planting beds consisted of a mixture of 40% cocopeat and 60% perlite. Planting was carried out in gutters with dimensions of $20 \times 30 \times 90$ cm, with three replications for each of the two fertilizer types and control treatments. Afterward, the planting beds were washed three times with tap water. During this stage,

Compound	Concentration of stock solution (g/L)	Volume of stock solution per liter of final solution (mL)	Element	Final concentration of element (ppm)
Macronutrients				
KNO3	101.10	6	Ν	224
Ca(NO ₃) ₂ -4H ₂ O	236.16	4	K	235
NH ₄ H ₂ PO ₄	115.08	2	Ca	160
MgSO ₄ -7H ₂ O	246.49	1	Р	62
-	-	-	S	32
-	-	-	Mg	29
Micronutrients	-	-	-	-
KCI	1.864	2	Cl	1.77
H ₃ BO ₃	0.773	2	В	0.27
MnSO ₄ -H ₂ O	0.169	2	Mn	0.21
ZnSO ₄ -7H ₂ O	0.288	2	Zn	0.13
CuSO ₄ -5H ₂ O	0.062	2	Cu	0.03
H2MoO4 (85% MoO3)	0.040	2	Mo	0.05
NaFeDTPA (10% Fe)	30.0	1	Fe	3

Table 1. The quantities of elements used in fertilizer production in accordance to Hoagland's formula.

the pH, TDS, and EC were recorded. Fertilization and irrigation were conducted utilizing three 200-liter tanks and tubes equipped with drip nozzles, delivering water at a rate of 0.3 L/min every other day. The concentration of humic acid and biochar in the solution was maintained at 1.5 g/L. Drinking water was used as a control for comparison. Continuous oxygenation in the tanks was ensured by employing a pump (HAILEA Model ACO 5505) that generated a flow within the fertilizers. This flow was instrumental in preventing the settling of the liquid fertilizer (prepared slurry). A total of 108 rooted Parus strawberry seeds were procured from Kurdistan, Iran, with careful attention given to selecting seeds of equal plant size and age. To avoid dormancy, the seeds were initially kept at a temperature of 4 °C for a period of 16 days. The seeds were dipped in a fungicide solution and promptly washed with water. Subsequently, 12 Parus strawberry seedlings were planted in the prepared bed, 14 cm apart from each other and at an appropriate depth in each gutter. To prevent plant stress and minimize damage, the seedlings were irrigated with drinking water for three days, three weeks after planting. Following two days of irrigation, the withered leaves were detached, resulting in the emergence of fresh leaves. Commencing on the 14th day, the plants were regularly fertilized at intervals of four days. Following every three fertilizations, the plants were thoroughly rinsed with drinking water to prevent the accumulation of fertilizer residue. Reproduction was facilitated manually by applying a gentle brush evenly to the flowers. The influence of each treatment on Parus strawberry yield was evaluated by gauging the plant growth parameters, including the size of the leaves, the mean number of fruits per plant, the maximal fruit weight, the fresh and dry fruit weights, as well as the average fresh fruit weight throughout the harvest period.

2.2. Statistical analysis

For this study, a completely randomized design with three replicates was employed, with each gutter representing one replicate. Data analysis was conducted using ANOVA in SPSS 22 software. Mean comparison at a significance level of 5% was determined using the Duncan test, and the standard error was taken into account during the analysis.

3. Results and discussion

The Parus variety was selected for the experiment because of its lower sensitivity to heat compared to other cultivars. The experiments measured the EC, TDS, and pH values of two types of biochar liquid fertilizers, as well as humic acid, Hoagland fertilizer, and urban water. The results are presented in **Table 2**.

Previous studies have indicated that biochar has the potential to increase pH levels due to its high concentration of calcium and magnesium^[26]. Liquid biochar fertilizer combined with humic acid, which contains organic sugar compounds that are converted by microorganisms to produce acetic or lactic acid, had

Table 2. Characteristics of two	types of fertilizers us	ed and urban water.
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Characteristic	Biochar fertilizer	Hoagland fertilizer	Urban water (control)
PH	8.50	7.90	7.2
EC (dS/cm)*	0.90	1.31	0.47
TDS (ppm)*	635	898	329

*dS/cm (deciSiemens per centimeter), ppm (part per million).

significantly higher levels than Hoagland solution and urban water, as shown in Table 2. The higher pH levels observed in the liquid biochar fertilizer combined with humic acid, as shown in Table 2, are beneficial for strawberry plant growth. Additionally, the biochar ash results in higher electrical conductivity (EC) compared to urban water. This can be attributed to the abundant presence of mobile K⁺ ions and the loss of volatiles during the biochar production process, which leads to an increase in elemental concentration^[27,28]. In the hydroponic system, the concentration of humic acid and the frequency of treatment can have an impact on tomato growth. Specifically, these factors have been found to increase root and branch length in tomato plants. However, it is worth noting that the effects of calcium and potassium elements can differ between leaves and fruits compared to roots and branches. This suggests that these elements may play distinct roles in different parts of the tomato plant, potentially influencing their development and characteristics^[29,30]. The application of liquid biochar fertilizer resulted in a noteworthy 5-day delay in the flowering of strawberry plants when compared to the use of Hoagland fertilizer. According to Table 3, Hoagland fertilizer led to a significant 5% increase in the number of fruits per plant compared to both liquid biochar and control fertilizers. However, it is worth noting that despite this difference, the employment of liquid biochar fertilizer exhibited a considerable enhancement in the fresh and dry weight of the fruit. This outcome suggests that the humic acid present in the biochar fertilizer positively influenced nutrient absorption, thereby contributing to improved fruit quality. Notably, the liquid biochar fertilizer sample demonstrated a significant increase in the overall weight of strawberries, implying substantial plant growth and, consequently, higher fruit yield when compared to other fertilizers.

Treatment	Number of fruits per bush in one harvest period	Maximum weight of strawberries (g)	Leaf area (cm²) per plant	Dry weight of fruit (g)	Fresh weight of fruit (g)	Total fresh weight of fruit in a harvest period (g)
Urban water (control)	$11.14^b\pm0.7$	$12.11^{\text{c}}\pm0.5$	$99^{\text{c}}\pm3.7$	$1.74^{\text{c}}\pm0.1$	$12.51^{\text{c}}\pm0.9$	$139.36^{\circ} \pm 6.3$
Liquid biochar fertilizer	$18.41^{\mathtt{a}} \pm 1.1$	$24.17^{\mathrm{a}}\pm0.6$	$192^{a}\pm4.9$	$2.61^{\text{a}}\pm0.2$	$18.76^{a}\pm1.3$	$345.37^a\pm9.1$
Hoagland fertilizer	$19.45^{\mathrm{a}}\pm0.9$	$19.12^b\pm0.8$	$170^{\text{b}}\pm2.3$	$2.09^{b}\pm0.3$	$15.03^{\text{b}}\pm1.2$	$292.33^b\pm7.2$

Table 3. The average yield of strawberry with biochar liquid mixed with humic acid, Hoagland fertilizer and urban water.

Different letters indicate significant differences at the P < 0.05 level with standard error (mean \pm SE).

Liquid biochar fertilizer application results in a 50% increase in both the fresh and dry weight of fruits when compared to the control group. However, the average fresh weight of fruits during harvest periods is higher than 50%. Additionally, using Hoagland chemical fertilizer leads to a 25% increase in both fresh and dry weight. The utilization of liquid biochar fertilizer results in a remarkable 65% increase in the number of fruits per plant during harvest periods when compared to the control group. Additionally, when considering the maximum fruit weight, the implementation of liquid biochar fertilizer demonstrates an impressive nearly 100% increase compared to the control group and a notable 27% increase compared to Hoagland's fertilizer. These findings highlight the significant positive impact of liquid biochar fertilizer on both fruit quantity and quality, indicating its effectiveness in promoting higher yields and larger, more robust strawberries.

The results strongly indicate that the application of liquid biological fertilizer or biochar can lead to a significant increase in fruit size and weight. In contrast, the observed decrease in fruit weight in the Hoagland chemical fertilizer treatment is likely attributed to the toxicity of specific elements, particularly nitrogen. This

is supported by the elevated levels of Total Dissolved Solids (TDS) and Electrical Conductivity (EC) reported in **Table 2**. These findings suggest that the use of chemical fertilizers such as Hoagland's can have adverse effects on fruit development, potentially impacting their overall quality and yield. On the other hand, the use of organic alternatives like liquid biological fertilizer or biochar offers a promising approach to promoting healthier and more substantial fruit production.

Humic acid has been recognized as a valuable plant biostimulant, as documented in previous research^[31]. It has shown consistent positive effects on plant growth and development. When it comes to strawberry cultivation, the impact of humic acid can vary depending on factors such as the specific type of humic acid used, dosage, and application methods. Numerous studies have reported various beneficial effects, particularly in terms of vegetative growth, yield, and fruit quality. These effects encompass factors such as mineral concentration, antioxidant compounds, and overall fruit quality. However, it is important to note that there is limited information available regarding the metabolic aspects influenced by humic acid, such as photosynthesis. Additionally, the research on postharvest fruit life and the plant's tolerance to pathogens is relatively limited. More studies are needed to delve deeper into these aspects and gain a comprehensive understanding of the potential benefits of humic acid in relation to photosynthesis, postharvest preservation, and pathogen resistance in strawberry cultivation^[32–34].

The utilization of liquid biochar fertilizer enriched with humic acid led to notable variations in fruit weights, demonstrating its efficacy as a growth enhancer. Conversely, a significant reduction in fruit weight was observed when employing the Hoagland chemical fertilizer treatment. The control sample, consisting of drinking water with a TDS value of 329 ppm, indicated the presence of some essential elements needed for the growth and fruiting of strawberries. The research findings strongly suggest that the tested fertilizer sample resulted in a greater harvest yield of fresh fruit. The study concludes that incorporating a biochar mixture derived from apple tree pruning, combined with humic acid, as an organic fertilizer in hydroponic cultivation, proves to be a highly effective approach for enhancing both the quality and quantity of fruit produced. Additionally, this method offers a practical means of utilizing garden pruning waste in an environmentally friendly manner.

The addition of biochar to the soil is considered one of the most effective practices for mitigating biotic stress and promoting increased crop productivity^[35]. Biochar has been found to have positive impacts on the intricate interactions between soil, plants, and water, resulting in enhanced photosynthetic performance as well as improved nitrogen and water use efficiency^[36]. To effectively mitigate threats and bolster agricultural production systems, it is crucial to enhance farmers' adaptive capacity and improve resilience and resource use efficiency^[37]. Conducting continuous assessments of physical, chemical, and biological factors is essential in this regard. These assessments should not only focus on agronomically relevant aspects but also delve deeper into understanding the mechanisms of action of biostimulant applications on plants. By doing so, novel techniques can be developed to enhance the nutraceutical quality of strawberries, increase fruit yield, and improve resistance against both biotic and abiotic stress factors.

4. Conclusion

The application of biostimulant products in strawberry cultivation has constantly been evolving over the years. Using a biochar mixture made from green waste of apple trees, along with humic acid, as an organic fertilizer for hydroponic cultivation is an efficient and environment-friendly method to enhance the quality and quantity of fruits while also effectively utilizing garden pruning waste. Hydroponic systems are a modern form of agriculture that allows plants to grow year-round. The study's results demonstrate that the incorporation of biochar contributes to increased fruit size and weight. In comparison to the control group, the sample grown with biochar showed a 65% increase in the number of fruits per plant. These findings highlight the effectiveness of biochar, in combination with humic acid, as an organic hydroponic fertilizer for enhancing both the quality

and quantity of strawberries. Efficient management of diverse fertilizers and methodologies in hydroponic greenhouse cultivation, a responsibility that should rank high on the agenda of scientists and engineers alike, is crucial for the protection of Earth's precious natural resources.

Author contributions

Methodology: SM; supervision: SM; conceptualization: MPF; investigation: MPF; data curation: MPF; project administration: SE; writing—original draft preparation: MSM; writing—review and editing: MM; All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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