Impact of Seaweed Extract and Biochar on the Morphological and Physiological Traits of *Petunia hybrida* L.

Hassan BAYAT[1] (✉)
Mohammad Javad VAHIDI[2]
Abdulghiyas RADAN[1]
Zahra PARSA[2]

Summary

Modern agriculture faces the challenge of declining soil organic matter, threatening future agricultural productivity. Consequently, alternative methods for enhancing soil stability and improving crop production are necessary. This study investigates the effects of biochar and seaweed extract on the growth, ornamental, and physiological characteristics of *Petunia hybrida* L. The experiment was conducted using a completely randomized design with four replications. The treatments included a control, barberry biochar at 2% by weight, seaweed extract at 1 ml/L, and a combination of seaweed extract and biochar. Results showed that the highest plant height and stem diameter were observed in plants treated with seaweed extract, showing increases of 24% and 25%, respectively, compared to the control. Flower longevity was significantly influenced, with the longest (6.75 days) and shortest (4.75 days) durations recorded in the seaweed extract and control treatments, respectively. Biochar and seaweed extract also affected the number of days until flowering, with the seaweed extract treatment resulting in the highest (135 days) and biochar treatment the lowest number (117 days). The seaweed extract treatment yielded the highest levels of chlorophyll a, b, total chlorophyll and carotenoids, with increases of 21%, 36%, 25%, and 25%, respectively, over the control. Additionally, the combination treatment of seaweed extract and biochar resulted in the highest levels of phosphorus, potassium, and calcium. The findings demonstrate that biochar and seaweed extract positively influence the growth, ornamental, and physiological traits of *Petunia hybrida* L., with seaweed extract proving to be more effective than biochar in improving these characteristics.

Key words

agricultural residues, barberry, flowering, nutrient dynamics

[1] Department of Horticultural Science, Faculty of Agriculture, University of Birjand, Birjand, Iran
[2] Department of Soil Science, Faculty of Agriculture, University of Birjand, Birjand, Iran

✉ Corresponding author: hassanbayat@birjand.ac.ir; hassanbayat55@gmail.com

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Introduction

Petunia hybrida L., a member of the *Solanaceae* family native to South America, comprises both annual and perennial cultivars. Petunia is a favoured seasonal flower widely used for ornamental purposes in urban green spaces and renowned for its low maintenance requirements and adaptable nature. The plant boasts an array of flower colours, including shades of purple, crimson, pink, red and white, rendering it invaluable within the floriculture industry (Farooq et al., 2021; Sahu et al., 2023).

Traditionally, agricultural residues, sewage sludge, urban waste, and animal manure have been utilized to augment soil organic matter. However, the direct application of these materials to agricultural lands has been associated with the generation of toxic substances and pathogens, necessitating their conversion into stable and benign organic products (Tang et al., 2024). Biochar has recently emerged as a promising solution in agriculture, serving as a source of organic matter conducive to plant growth and soil improvement (Ebrahimi et al., 2022; Ngambia et al., 2024). Produced through heat treatment of various biomasses like wood chips, animal manure and plant residues under limited oxygen conditions, biochar presents significant agricultural and ecological advantages (You et al., 2021; Yan et al., 2022; Vahidi et al., 2023). Its integration into agricultural soil facilitates carbon sequestration, nutrient retention, enhancement of soil structure, stimulation of microbial activity, improvement of water retention, acidity reduction, pollutant mitigation, and creation of favourable growth environments (Ajoy et al., 2022).

In Iran's South Khorasan province, extensive cultivation of barberry results in substantial annual waste production (Vahidi, 2020). Innovating methods for waste utilization become imperative in this context. Barberry branch and leaf waste, if promptly converted into biochar post-harvest, could represent a sustainable solution (Gao et al., 2020; Vahidi et al., 2022). Aligned with principles of sustainable agriculture, soil fertility, and plant nutrition, they constitute pivotal aspects in fostering plant growth (Al-Wabel et al., 2021; Mak-Mensah et al., 2021). Studies attest to biochar's positive impacts on soil fertility, crop yield, and quality. For instance, cherry biochar at 2% and 3% concentrations significantly enhances growth parameters in basil (*Ocimum basilicum* L.), while rice husk biochar augments ornamental parameters in African marigold (*Tagetes erecta* L.) (Jabborova et al., 2021b; Reddy et al., 2023). Biochar supplementation enriches the soil with essential nutrients, thereby promoting the growth of medicinal plants like senna (*Cassia angustifolia* Vahl.) (Ajoy et al., 2022). Furthermore, the utilization of seaweed extracts has gained traction in organic and sustainable agriculture. Seaweeds, abundant along beaches globally, serve as multifaceted resources for food, feed, biofuels, fertilizers and pharmaceuticals (Arab et al., 2022; Chandimali et al., 2023). Liquid seaweed extracts, employed through foliar spraying, significantly enhance plant photosynthetic capacity, stress tolerance, soil microbial activity and nutrient availability (El Boukhari et al., 2020; Ahmed, 2022; Ali et al., 2022; Arab et al., 2022).

Despite the manifold benefits of biochar and seaweed extract, their application in the floriculture industry remains relatively unexplored. Moreover, comprehensive investigations into the efficacy of barberry biochar as an organic fertilizer for petunia cultivation are lacking. Hence, this study endeavours to evaluate the effects of biochar and seaweed extract on the growth, ornamental characteristics and physiological responses of *Petunia hybrida* L., thus contributing to a deeper understanding of sustainable cultivation practices in ornamental horticulture.

Materials and Methods

To explore the impact of biochar derived from barberry residues and seaweed extract on the morphological, physiological and biochemical attributes of *Petunia hybrida* L., we conducted a completely randomized experiment with four replications at the research greenhouse and laboratory of the Faculty of Agriculture, University of Birjand, in 2022.

Experimental Treatments

The experimental treatments were: control (no biochar or seaweed extract); barberry biochar at 2% by weight; seaweed extract at a concentration of 1 ml/litter and combination of 2% barberry biochar and 1 mL·L^{-1} seaweed extract.

Each replication comprised four pots, each housing one Petunia plant. Sixteen pots, measuring 14 cm in diameter and height, were utilized for the experiment. Before use, the soil underwent air-drying and sieving through a 2 mm sieve. Soil physical and chemical properties were assessed following standard methods (Wilke, 2005) (Table 1).

Petunia hybrida L. seeds were sourced from Poponik Company, Tehran, Iran. Initially, the seeds were sown in 105-hole seedling trays (2.3 cm diameter, 5 cm height) filled with a mixture of cocopeat and peat (2:1 ratio). After 45 days of germination, one seedling was transplanted into each pot. Irrigation was conducted biweekly at a rate of 250 ml per pot, adjusted based on plant requirements. Acadian seaweed extract containing *Ascophyllum nodosum* seaweed extract (Acadian Seaplants Co, Canada) was foliar-sprayed four times at 10-day intervals, with the first spray administered 14 days post-transplanting.

Barberry biochar was prepared from pruned barberry leaves and branches. Following air-drying and crushing, the samples were oven-dried at 105 °C for 24 hours. Subsequently, they underwent further crushing and thermal decomposition at 300 °C for 2 hours under limited oxygen conditions to produce biochar. The resultant biochar was sieved through a 2 mm sieve, and 2% biochar by weight was added to the designated treatments (Table 1).

Plant growth was monitored every 10 days, commencing 14 days post-seedling transplantation. Following the experiment (155 days post-seed cultivation), growth, reproductive, and physiological parameters were evaluated.

Measurement of Total Chlorophyll *a*, *b*, and Leaf Carotenoids

Chlorophyll and carotenoid content were determined following Arnon's method (Arnon, 1947). Fresh plant leaves (0.5 g) were homogenized with liquid nitrogen in a mortar and pestle. The homogenate was mixed with 20 mL of 80% acetone, and centrifuged at 600 rpm for 10 minutes, and absorbance was measured at 470, 645, 663 nm using a spectrophotometer (Model Unico 2100, China).

Table 1. The physical and chemical properties of the soil and barberry

	Initial barberry	Barberry biochar	Soil
Oxygen %	22.6	11.8	
pH	6.55	8.8	7.9
Sp			27
EC (mmho/cm)	6.5	4.3	2.3
Hydrogen (%)	7.8	2.2	
Nitrogen (%)	0.48	0.85	0.78
Phosphorous (%)	0.18	0.58	13.8
Calcium (%)	2.11	5.24	5.13
Potassium (%)	0.34	0.67	0.53
Magnesium (%)	0.07	0.15	2.53
Ca+Mg	2.18	5.39	8
C/N	112.92	84.2	
OC (%)	54.2	78.57	
Sodum (%)	0.05	0.1	
BD	0.53	0.65	1.81
%BP		31	2.65
%AB		14.5	
Sand			76
Silt			18
Clay			6
Organic matter			0.2
Textural			Loamy Sand

Note: BP - biochar performance, AB - ash biochar, pH - soil reaction, EC - electrical conductivity, OC - organic carbon, C/N - carbon to nitrogen ratio, BD - bulk density, Sp: saturation percentage, Ca+Mg - calcium+ magnesium

Determination of Nutrient Concentration

To determine nitrogen, phosphorus, potassium, calcium, and magnesium concentrations, plant leaves at the flowering stage were collected from each treatment. Samples were oven-dried at 70 °C for 48 hours, powdered and digested for phosphorus, potassium, calcium and magnesium using concentrated hydrochloric acid via the dry digestion method. Nitrogen was determined using the Kjeldahl method. The concentration of each nutrient was analysed using appropriate spectrophotometric and titrimetric techniques (Jones, 2005).

Data Analysis

Data were analysed using JMP 13 software, and graphical representations were generated using Excel. Mean comparisons were conducted using the LSD test at a significance level of $P < 0.05$.

Results

Growth and Reproductive Traits

The influence of biochar fertilizer and seaweed extract treatments on various traits of *Petunia hybrida* L. was examined through analysis of variance, revealing significant effects (Table 2). Leaf length, stem diameter, root volume and fresh and dry weight of root displayed significance at a probability level of 1%, while plant height, leaf width, root length, shoot fresh weight and total dry weight were significant at a five percent probability level. Comparative analysis indicated substantial increases in leaf length and width, notably with the biochar + seaweed extract treatment showing a 45% increase in leaf length and a 22% increase in leaf width compared to the control (Table 2). Plants treated with seaweed extract exhibited the highest plant height and stem diameter, with increments of 24% and 25%, respectively, compared to the control. Root length did not significantly differ between treatments, although biochar + seaweed extract application led to reduced root length compared to other treatments. Root volume increased by 37% and 42% with seaweed extract and biochar + seaweed extract treatments, respectively, compared to the control (Table 2).

Seaweed extract and biochar applications enhanced root weight, particularly with the biochar + seaweed extract treatment, resulting in a 28% and 40% increase in fresh and dry root weight, respectively, compared to the control (Table 3). Seaweed extract treatment also led to a 4% increase in fresh weight of aerial parts compared to the control. Total dry weight did not significantly differ between seaweed extract and biochar treatments compared to the control (Table 3). Additionally, seaweed treatment exhibited the highest number of leaves during the observation period of experiment (Fig. 1).

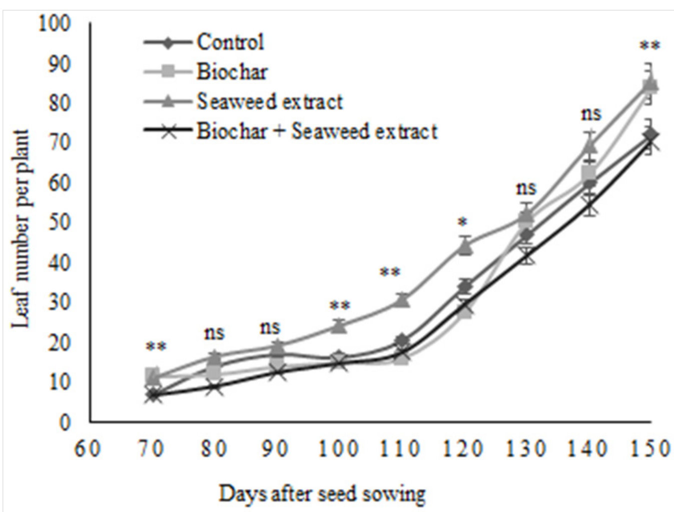

Figure 1. Leaf count per plant across different treatments over the observation period (*, **, ns: Significance at at $P < 0.05$ and $P < 0.01$ probability levels and non-significance, respectively)

Table 2. Effects of barberry biochar, seaweed extract and their combination on the growth parameters of petunia (*Petunia hybrida* L.)

Treatment	Leaf length (cm)	Leaf width (cm)	Plant height (cm)	Stem diameter (mm)	Root length (cm)	Root volume (cm^3)
Control	7.37^{c}	2.87^{b}	41.25^{b}	4.52^{c}	27.00^{ab}	22.75^{b}
Biochar	8.75^{b}	3.50^{a}	42.5^{b}	4.95^{b}	29.00^{a}	23.00^{b}
Seaweed extract	8.25^{bc}	2.62^{b}	51.25^{a}	5.65^{a}	29.50^{a}	31.25^{a}
Biochar + Seaweed extract	10.75^{a}	3.12^{ab}	45.01^{ab}	4.89^{b}	25.25^{b}	32.50^{a}
Significance level	**	*	*	**	*	**

Note: *, **, ns: Significance at at $P < 0.05$ and $P < 0.01$ probability levels and non-significance, respectively; Means in the columns followed by the same letter are not significantly different according to the LSD test at $P < 0.05$

Table 3. Effects of barberry biochar, seaweed extract and their combination on the dry weights of root and shoot of petunia (*Petunia hybrida* L.)

Treatment	Root fresh weight (g)	Root dry weight (g)	Fresh weight of shoot (g)	Dry weight of shoot (g)	Total dry weight (g)
Control	22.77^{b}	2.03^{c}	67.43^{bc}	26.80^{a}	28.83^{ab}
Biochar	21.70^{b}	2.07^{c}	66.69^{c}	26.07^{a}	28.14^{b}
Seaweed extract	26.87^{a}	2.52^{b}	70.08^{a}	27.27^{a}	29.81^{a}
Biochar + Seaweed extract	29.36^{a}	2.85^{a}	69.74^{ab}	27.28^{a}	30.13^{a}
Significance level	**	**	*	ns	*

Note: *, **, ns: Significance at $P < 0.05$ and $P < 0.01$ probability levels and non-significance, respectively; Means in the columns followed by the same letter are not significantly different according to the LSD test at $P < 0.05$

Reproductive Traits

Significant impacts of biochar fertilizer and seaweed extract treatments on the longevity of flowers and days to flowering were observed at a 1% probability level, while flower diameter remained unaffected (Table 4). The longest flower durability (6.75 days) was observed in plants treated with seaweed extract, whereas the shortest durability (4.75 days) was in the control. Days to flowering ranged from 117 days in the biochar treatment to 135 days in the seaweed extract treatment (Table 4). Seaweed and control treatments demonstrated the highest and lowest number of flowers during the observation period, respectively (Fig. 2).

Photosynthetic Pigments

The analysis of variance indicated significant effects of seaweed extract and biochar treatments on photosynthetic pigment traits, including chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids, all at a 1% probability level (Table 5). Seaweed extract treatment exhibited the highest levels of chlorophyll *a*, *b*, and total chlorophyll, with increases of 21%, 36%, and 25%, respectively, compared to the control. Additionally, the highest carotenoid levels were observed in the seaweed extract treatment, showing a 25% increase compared to the control (Table 5).

Table 4. Effects of barberry biochar, seaweed extract and their combination on the flowering of petunia (*Petunia hybrida* L.)

Treatment	Flower diameter (cm)	Flower durability (day)	Days to flowering
Control	6.5^{a}	4.75^{c}	125.25^{b}
Biochar	5.87^{a}	5.75^{b}	117.00^{c}
Seaweed extract	5.75^{a}	6.75^{a}	135.00^{a}
Biochar + Seaweed extract	5.74^{a}	5.75^{b}	125.25^{b}
Significance level	ns	**	**

Note: *, **, ns: Significance at $P < 0.05$ and $P < 0.01$ probability levels and non-significance, respectively; Means in the columns followed by the same letter are not significantly different according to the LSD test at $P < 0.05$

Table 5. Effects of barberry biochar and seaweed extract and their combination on the leaf chlorophyll and carotenoid content of petunia (*Petunia hybrida* L.)

Treatment	Chlorophyll a (mg g FW^{-1})	Chlorophyll b (mg g FW^{-1})	Total chlorophylls (mg g FW^{-1})	Carotenoids (mg g FW^{-1})
Control	0.64^{b}	0.25^{c}	0.89^{c}	0.24^{b}
Biochar	0.66^{b}	0.26^{bc}	0.93^{bc}	0.23^{b}
Seaweed extract	0.78^{a}	0.34^{a}	1.12^{a}	0.29^{a}
Biochar + Seaweed extract	0.70^{b}	0.30^{ab}	1.01^{b}	0.25^{b}
Significance level	**	**	**	**

Note: *, **, ns: Significance at %5 and %1 probability levels and non-significance, respectively; Means in the columns followed by the same letter are not significantly different according to the LSD test at $P < 0.05$.

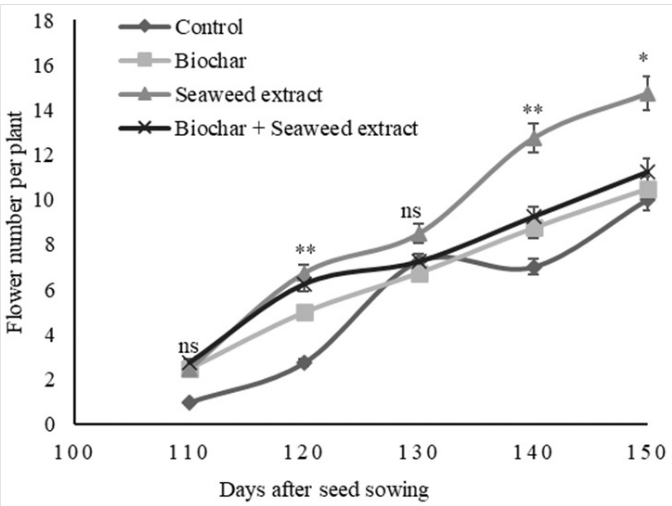

Figure 2. Flower count per plant across different treatments over the observation period. (*, **, ns: Significance at at $P < 0.05$ and $P < 0.01$ probability levels and non-significance, respectively).

Nutrient Content

Biochar and seaweed fertilizers significantly affected the content of calcium, magnesium, potassium and phosphorus, while nitrogen content remained unaffected (Table 6). Seaweed extract + biochar treatment showed the highest percentage of phosphorus, potassium and calcium compared to other treatments and the control. The highest magnesium content was observed in the seaweed extract treatment, demonstrating significant differences between other treatments and the control (Table 6).

Discussion

This study highlights the positive impact of biochar and seaweed extract treatments on the vegetative and ornamental parameters of *Petunia hybrida* L. revealing significantly enhanced growth and flowering compared to the control. The most robust outcomes were achieved with the combined biochar and seaweed extract treatment, as well as the seaweed extract alone. The growth-stimulating properties of seaweed, attributed to various bioactive compounds, are well-documented (El Boukhari et al., 2020). Seaweed extract serves as a rich nutrient source, containing amino acids, vitamins, antioxidants and natural hormones that facilitate the slow release of nutrients, enhancing overall plant performance (Singh et al., 2015). The efficacy of seaweed extract in promoting flowering traits has been demonstrated in various plants such as daffodils, cucumbers and tomatoes (Shahira, 2015; Di Stasio et al., 2018; Sharma et al., 2019). The natural growth hormones in seaweed extract, including auxin and cytokinin, stimulate cell division and enlargement, leading to improved plant growth and flowering (Lall et al., 2017). Biochar's positive influence on plant growth parameters is consistent with previous research. Studies on *Brassica chinesnis* and *Lactuca sativa* L. have shown that rice

Table 6. Effects of barberry biochar and seaweed extract and their combination on the leaf nutrient content of petunia (*Petunia hybrida* L.)

Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
Control	2.37^{a}	0.16^{b}	2.15^{c}	1.67^{b}	0.99^{c}
Biochar	2.27^{a}	0.20^{b}	2.82^{b}	1.92^{ab}	1.36^{b}
Seaweed extract	2.15^{a}	0.19^{b}	2.62^{b}	1.75^{b}	1.72^{a}
Biochar + Seaweed extract	2.21^{a}	0.28^{a}	3.56^{a}	2.12^{a}	0.51^{d}
Significance level	ns	**	**	*	**

Note: *, **, ns: Significance at %5 and %1 probability levels and non-significance, respectively; Means in the columns followed by the same letter are not significantly different according to the LSD test at $P < 0.05$.

husk biochar increases total dry weight, root dry weight, plant height and stem number compared to controls (Carter et al., 2013). Biochar application has also been linked to increased leaf dimensions in basil and improved growth indicators in perennial ryegrass (Jabborova et al., 2021b; Safari et al., 2023). Additionally, biochar enhances ornamental parameters such as flower number, diameter, longevity and flowering period in African parsley (Reddy et al., 2023). Biochar improves soil permeability and water retention, thereby increasing the availability of moisture and nutrients essential for plant growth (Fedeli et al., 2024).

The significant increase in leaf chlorophyll content observed in this study aligns with previous findings associating biochar application with enhanced photosynthetic activity and biomass production (Zhang et al., 2022). Other studies have reported increased chlorophyll content in plants like evening primrose and *pelargonium* with plant waste biochar (Altaf et al., 2021), and chlorophyll and carotenoid content in spinach with cherry biochar application (Jabborova et al., 2021a). Biochar's ability to enhance moisture retention improves plant water relations, while seaweed extract's high nutrient content further boosts nutrient uptake, promoting overall plant health and growth (Chandimali et al., 2023). The presence of betaine in seaweed extract, which increases photosynthetic pigment concentration and chlorophyll levels, also contributes to improved plant growth and development (Manimaran et al., 2018).

The significant increase in leaf nutrient content of petunia plant was obtained in this study. Jabarova et al (2021a) reported that biochar improves nutrient content such as nitrogen, phosphorus and potassium in basil plants. The amount of nitrogen, potassium, magnesium and phosphorus of tomato leaves has increased with the use of biochar (Vaccari et al., 2015). Combining seaweed extract and biochar provides a rich source of nutrients, enhancing plant performance by improving soil characteristics and nutrient availability (Karthik et al., 2023; Usman et al., 2023). These organic amendments not only enrich soil organic matter but also improve its physical, chemical and biological properties, thereby boosting plant growth and development.

Conclusion

The results of this study demonstrate that the application of biochar fertilizers and seaweed extract significantly enhances the growth, ornamental qualities and physiological traits of *Petunia hybrida* L. Among the two treatments, seaweed extract proved to be more effective than biochar in improving these traits. These findings underscore the potential of organic fertilizers as effective tools for enhancing plant growth and flowering. Incorporating these organic amendments can significantly contribute to sustainable agricultural practices, promoting healthier plant development while maintaining environmental integrity. Future research should explore the long-term impacts and optimization of these treatments to fully harness their benefits in various agricultural settings. Additionally, investigating the underlying mechanisms by which biochar and seaweed extract influence plant physiology could provide deeper insights, facilitating their broader application in agriculture.

CRediT Authorship Contribution Statement

Hassan Bayat, Abdulghiyas Radan, Zahra Parsa: Material preparation, data collection and analysis. **Mohammad Javad Vahidi:** Writing the first draft of the manuscript. All authors contributed to the study conception and design and read the manuscript and approved the final version of it.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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