Assessment of nutrient content, leaf photosynthesis pigments, and growth of blue fescue in biochar-amended soil and humic acid in order to use in landscape

Azadeh Mousavi Bazaz*, Yahya Selahvarzi, Sana Ansari
1. Department of Horticultural science and landscape architecture, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

---

Abstract

The effects of different levels of biochar and humic acid on the growth parameters, photosynthetic pigments, and nutritional content of blue fescue were investigated. The experimental design of this study was a factorial experiment based on a completely randomized design with three replications. The results showed that the interaction between humic acid and biochar had a significant effect on shoot length, shoot dry biomass, root-to-shoot (R/S) biomass ratio, total chlorophyll, chlorophyll a, chlorophyll b, carotenoids, and leaf concentrations of N, P, K, Ca, Mn, Fe, Zn, and Cu. Root dry biomass was affected by humic acid and increased at 200 mg L^{-1} and 400 mg L^{-1} humic acid compared to the control. Increasing the application rates of biochar and humic acid resulted in an increase in leaf nutrient contents in blue fescue. The highest levels of chlorophyll a and chlorophyll b were recorded at 40 g kg^{-1} biochar combined with 200 mg L^{-1} humic acid and 40 g kg^{-1} biochar combined with 400 mg L^{-1} humic acid, respectively. The use of biochar and humic acid, especially at application rates of 40 g kg^{-1} and 400 mg L^{-1}, respectively, significantly improved most of the traits evaluated in this experiment. When biochar and humic acid were applied together, their effects were more pronounced.

Keywords: Nutrition, Ornamental grass, Festuca glauca, Plant growth, Chlorophyll

Mousavi Bazaz, A. Y. Selahvarzi, S. Ansari, 2024. Assessment of nutrient content, leaf photosynthesis pigments, and growth of blue fescue in biochar-amended soil and humic acid in order to use in landscape. *Iranian Journal of Plant Physiology 14* (3), 5063- 5073.

Introduction

In crop production, selecting the appropriate growing media for propagation and providing healthy seedlings and shrubs for use in landscaping and horticulture is essential. Plants provided with suitable growing media can tolerate abiotic stress (Fornes and Belda, 2019). The presence of nutrients available to plants depends on the nutrient concentration of the substrate (Zhang et al., 2014). In recent decades, biochar has attracted significant attention because of its practical benefits, such as its rich carbon content, high cation exchange capacity (CEC), and improved soil stability (Nobile et al., 2020).

Biochar as a soil amendment has various effects on crop growth and quality, soil nutrient content, and soil microbial community (Chen et al., 2018). Biochar reduces sodium (Na) absorption and increases potassium (K) absorption; it also improves productivity, biomass, and photosynthesis under abiotic stress conditions (Ali

---

** Corresponding Author*
E-mail Address: mousaviba@um.ac.ir

Received: January, 2024
Accepted: April, 2024

et al., 2017). Furthermore, it improves the physical, biochemical, and biological properties of the soil (Chen et al., 2018). Biochar is produced from biomass sources, including animal manure, crop, and garden waste, through thermal decomposition of biomass under oxygen-limited conditions (Zoghi et al., 2019). In some regions, the availability of nutrients such as manganese (Mn), phosphorus (P), zinc (Zn), iron (Fe), and copper (Cu) is low due to high soil pH values. In such soils, the use of organic materials such as biochar can affect nutrient concentrations and eventually increase plant growth (Aboukila et al., 2018). Biochar has been shown to have a positive impact on production quality (Akhtar et al., 2014). Similarly, in controlled environments, growth parameters increase with the addition of biochar in the field (Major et al., 2010).

The response of crop species to biochar varies (Fascella et al., 2020). For example, poinsettia plants grown with 0% and 40% biochar exhibited similar agronomic yields (Guo et al., 2018). The growth of Euphorbia x lomi was lower in plants grown with 100% peat compared to a mixture of 40% peat and 60% biochar (Dispenza et al., 2016). Furthermore, some studies indicate that nutrient concentrations in biochar may not affect soil nutrition (Nobile, Denier, and Houben, 2020). For example, biochar significantly reduces nitrogen (N) content in soil (Agbna et al., 2017). However, other studies have reported opposite results (Chen et al., 2018).

Currently, the use of organic acids is common in crops and horticultural products to improve their quantitative and qualitative characteristics. One of these compounds is humic acid (Khorasaninejad et al., 2018). Humic acids have a positive effect on plant nutrient uptake and are important for the availability of micronutrients in plants (Nikbakht et al., 2008). This can enhance plant nutrient uptake by improving root growth, which increases surface area and facilitates more efficient nutrient uptake (Nikbakht et al., 2008). Organic matter can contribute directly to soil nutrient storage or indirectly impact nutrient bioavailability by altering soil biochemical properties. Therefore, it can positively influence soil physical properties and fertility (Karimi et al., 2020).

Ornamental grasses are low-maintenance, low-water plants that are commonly used in green spaces (Jarecka and Sosnowski, 2021). Festuca glauca Lam. is an ornamental grass that forms a compact cluster of narrow, long silver-blue leaves. It is widely grown, primarily in rock gardens and perennial garden beds (Yuan et al., 2011).

While some researchers have documented the effect of biochar on plant growth, leaf nutrient content, water holding capacity, etc., in various field crops, there have been no reports on the effects of biochar and humic acid on Festuca glauca. The aim of this study is to assess the effect of biochar and humic acid addition on blue fescue growth response, photosynthetic pigment content, and nutritional components. The outcomes of this research could be used to enhance the growth of blue fescue plants after transplanting and in green spaces.

Materials and Methods

Plant material, biochar and soil condition

The experiment was carried out from October to November 2022 under greenhouse conditions (27 °C Day/18 °C Night and 60% Humidity) at the Faculty of Agriculture of Ferdowsi University of Mashhad, Iran. The biochar (BC) used in this study was prepared from the woody branches of pistachio (Pistacia vera L.). Slow pyrolysis was carried out at a temperature of 500 °C. The wood branches were placed in an electric vacuum furnace and the slow pyrolysis process lasted five hours. Some properties of biochar are given in Table 1.

A sandy loam soil with 42% sand, 36% silt, and 22% clay was selected. The soil texture assessed by the hydrometric method (GeeandBauder, 1986). Electrical conductivity, cation exchange capacity, pH, calcium carbonate equivalent, available P, and organic matter are measured by standard methods (Sparks et al., 2020). The total nitrogen concentration of soil was determined by the Kjeldahl method (BremnerandHauck, 1982). The ammonium acetate (NH4OAc) extraction technique was applied to assess the available potassium (K) (Chen et al., 2018).

Table 1
Physical and chemical properties of pistachio BC

Parameter	Biochar
pH	7.2
EC (ds/m)	4.2
N (%)	6.5
P (mg kg-1)	0.074
K (mg kg-1)	0.226
Ca (ppm)	51074.3
Mg (%)	0.036
Cu (ppm)	72.147
Mn (ppm)	240.947
Zn (ppm)	171.582
Fe (ppm)	22021.3
Organic Carbon (%)	7.41
Organic matter (%)	12.77
CEC (meq. 100 g−1)	6.3
Bulk density (g cm-3)	0.39

The total nitrogen (N) content was 2108 ppm, while P and K contents were 10.82 and 66 mg kg−1, respectively; pH was 7.8; cation exchange capacity (CEC), 8.64 meq. 100 g−1, EC was 4.5 ds m-1 and organic matter 0.175%.

After air-drying, the soil was passed through a sieve with a mesh size smaller than 2mm. Then, the different levels of biochar were mixed uniformly with the soil, the levels of biochar were 0, 20, and 40 g kg-1 of dry soil. Plastic container size 12 cm in diameter filled with a prepared mixture of soil and biochar. Three one-year-old-plants of blue fescue (Festuca glauca) transplanted into containers. The containers kept in a greenhouse condition. The humic acid (HA) (Plant, s Choice Company from USA) used in the experiment with irrigation water in three levels: 0, 200 and, 400 mg L-1 every 20 days until the end of the experiment. Plants kept for 70 days in greenhouse conditions and at the end of the experiment, some parameters measured.

Growth measurements

Crown diameter, maximum shoot length and maximum root length were recorded at the end of the experiment. Shoot and root dry biomass were also recorded. The shoot and root separately dried at 60 °C for 48 h. Plant height and dry weight were measured by a ruler and a precision Lab balance (GF-300), respectively. The root-to-shoot dry biomass (R/S biomass) was also calculated.

Mineral and photosynthesis pigments assessment

For assessment of nutrient content, shoots were cut and washed with distilled water and dried at 60 °C for 48 h. The dried shoot biomass was milled and passed through a 0.5-mm sieve. To quantify the elements, two grams of leaf sample was digested in 2 mol hydrochloric acid after heating for four h at 550 oC (Zoghi et al., 2019). The P content was measured by the ascorbic acid method using a UV-vis spectrophotometer (MurphyandRiley, 1962). The K content of plant samples was analyzed using a flame photometer (Karimi et al., 2020). Leaf N content was determined using the Kjeldahl method (BremnerandHauck, 1982). Plant available from calcium (Ca), zinc (Zn), iron (Fe), copper (Cu), and manganese (Mn) using DTPA extracted and measured using atomic absorption spectroscopy (Kazemi et al., 2019). Leaf chlorophyll and carotenoids were extracted by acetone 80% and measured (Lichtenthaler, 1987).

Experimental design

Data analysis were carried out in a factorial experiment based on completely randomized design with three replications. Two factors comprised of three levels of biochar (0, 20, and 40 g kg-1 of dry soil) and three levels of humic acid (0, 200 and, 400 mg L-1). Data analysis and mean comparison was determined by LSD test at p≤0.05 using SAS 9.4 software.

Results

The results showed that the interaction between humic acid and biochar had a significant effect on shoot length, shoot dry biomass, root-to-shoot (R/S) biomass ratio, total chlorophyll, chlorophyll a, chlorophyll b, carotenoids, and leaf concentrations of N, P, K, Ca, Mn, Fe, Zn, and Cu (Table 2).

Plant Growth Parameters

According to these outcomes, the combined use of biochar (BC) and humic acid (HA) was significant at the 5% probability level for shoot dry biomass. The highest shoot dry biomass was observed with the

Table 2
Analysis of variance mean square effects of pistachio biochar (BC) and humic acid (HA) on some morphological, physiological and nutrient parameters of blue fescue.

Sources of variation	df	Shoot length	Root length	Root dry biomass	Shoot dry biomass	R/S biomass	Crown diameter	Chlorophyll a
BC	2	1.02*	2.20 ns	5.91*	4.93**	0.56**	26.78**	0.16*
HA	2	1.08*	2.68 ns	5.16*	0.30 ns	0.17*	4.22 ns	0.06 ns
BC*HA	4	2.82**	7.99 ns	1.68 ns	0.53*	0.02*	3.99 ns	0.27**
Error	18	4.69	54.13	18.74	4.71	0.12	65.51	0.75
CV (%)		3.16	8.37	17.91	10.01	21.91	8.84	1.93

Sources of variation	df	Chlorophyll b	Total Chlorophyll	Carotenoid	N	p	K	Mn
BC	2	0.30 ns	1.40**	0.032**	0.22**	0.00005*	0.59**	87.05**
HA	2	0.22 ns	0.78 **	0.11**	0.22**	0.0001**	0.30*	66.12**
BC*HA	4	0.61*	0.70**	0.04**	0.20**	0.0004**	0.23*	230.45**
Error	18	2.63	0.85	0.07	0.17	0.0002	1.09	51.84
CV (%)		4.96	1.19	4.35	5.43	7.15	5.91	4.33

Sources of variation	df	Ca	Fe	Zn	Cu
BC	2	246471.62 ns	27979.43**	248.35*	3.87*
HA	2	3051774.43**	17475.43 **	234.51*	2.48 ns
BC*HA	4	3571586.15 **	20833.19**	308.88**	6.35**
Error	18	3161981.21	3294.05	1140.00	17.71
CV (%)		6.56	6.66	15.99	24.61

CV: Coefficient of Variation; ns, *, **: not significant, significant difference at $P < 0.5$ and $P < 0.01$ respectively

combined application of 200 mg L^{-1} HA and 40 g kg^{-1} BC (Fig. I. a). There was also a significant difference between 40 g kg^{-1} BC and 0 g kg^{-1} BC in all levels of HA (Fig. I. a).

The best result for shoot length was recorded with 20 g kg^{-1} BC and 400 mg L^{-1} HA and 20 g kg^{-1} BC and 200 mg L^{-1} HA, respectively (Fig. I. b). The R/S biomass increased in plants grown in substrates combined with BC and HA, but in all levels of BC, the highest R/S biomass was recorded with 0 mg L^{-1} HA (Fig. I. c).

The interaction effect was insignificant for crown diameter and root dry biomass (Table 1). Root dry biomass was affected by HA and increased with 200 and 400 mg L^{-1} HA compared to 0 mg L^{-1} HA and showed a significant difference from this level of HA (Fig. II. b). Conversely, biochar had no positive effect on this trait (Fig. II. a). Crown diameter was significantly higher at 20 g kg^{-1} BC compared to other levels, with the highest value at this level (Fig. II. c). In this study, humic acid addition showed a more significant effect on root growth compared to the control (Fig. II. b).

Nutrient Concentration

Both macro and micronutrients were affected by biochar amendment and humic acid. Increasing biochar (from 0 g kg^{-1} to 40 g kg^{-1}) and HA (from 0 mg L^{-1} to 400 mg L^{-1}) resulted in an increase in leaf phosphorus in blue fescue (Fig. III. a). The highest phosphorus content was recorded at 40 g kg^{-1} BC with 400 mg L^{-1} HA and 20 g kg^{-1} BC with 400 mg L^{-1} HA, respectively, which showed significant differences from the control. In all BC levels, the lowest phosphorus content was observed at 0 mg L^{-1} HA (Fig. III. a). In contrast, leaf potassium (K) content in BC levels decreased with increasing HA; except at 20 g kg^{-1} BC with 400 mg L^{-1} HA, leaf K values decreased with increasing HA. The highest leaf K was recorded at 40 g kg^{-1} BC with 0 mg L^{-1} HA (Fig. III. b). Regarding nitrogen (N), the interaction of BC and HA appeared to have a negative effect, with leaf N decreasing at 20 and 40 g kg^{-1} BC with increasing HA (Fig. I. c). The

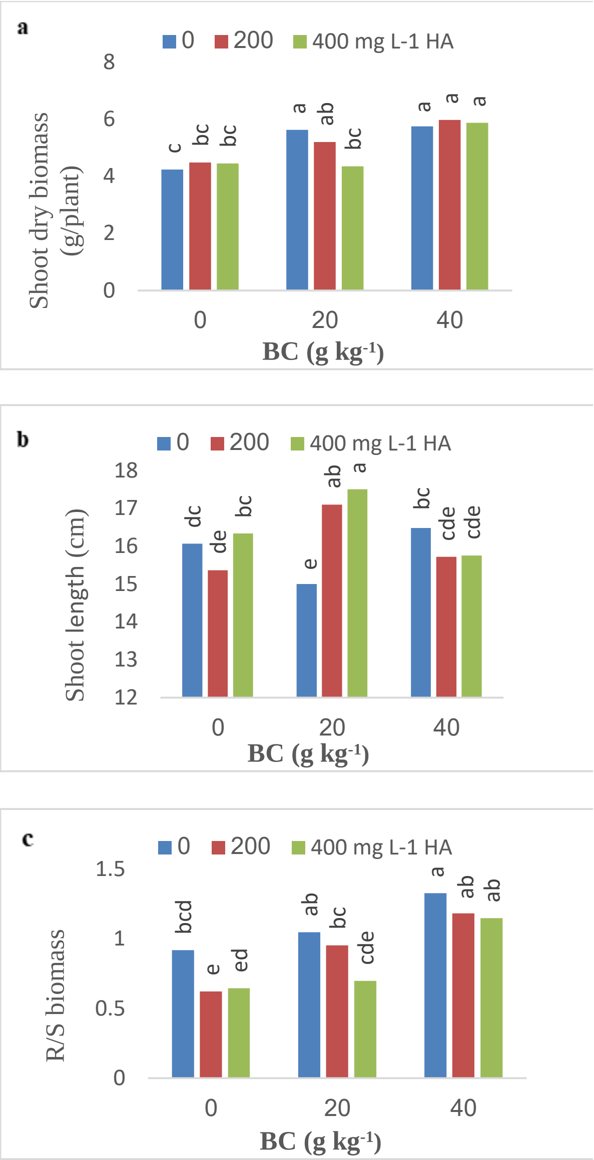

Fig. I. Effect of biochar and humic acid on Shoot dry biomass (a), Shoot length (b) and R/S biomass (c) of blue fescue. Different letters are significant at $p < 0.05$ (LSD test).

highest N content was observed at 20 g kg^{-1} BC with 0 mg L^{-1} HA (Fig. III. c).

Calcium (Ca) concentration was positively affected by the addition of biochar and humic acid, with the highest values recorded at 40 g kg^{-1} BC with 400 mg L^{-1} HA (Fig. III. d). Leaf iron (Fe) and copper (Cu) significantly increased with increasing BC and HA, with the highest leaf Fe recorded at 40 g kg^{-1} BC with 400 mg L^{-1} HA (Fig. III. e and III. h). At 20 and 40 g kg^{-1} BC, leaf zinc (Zn) increased compared to 0 g kg^{-1} BC (Fig. III. f). Although the highest manganese (Mn) concentration was observed at 40 g kg^{-1} BC with 400 mg L^{-1} HA, there was no noticeable difference among other treatments (Fig. III. g).

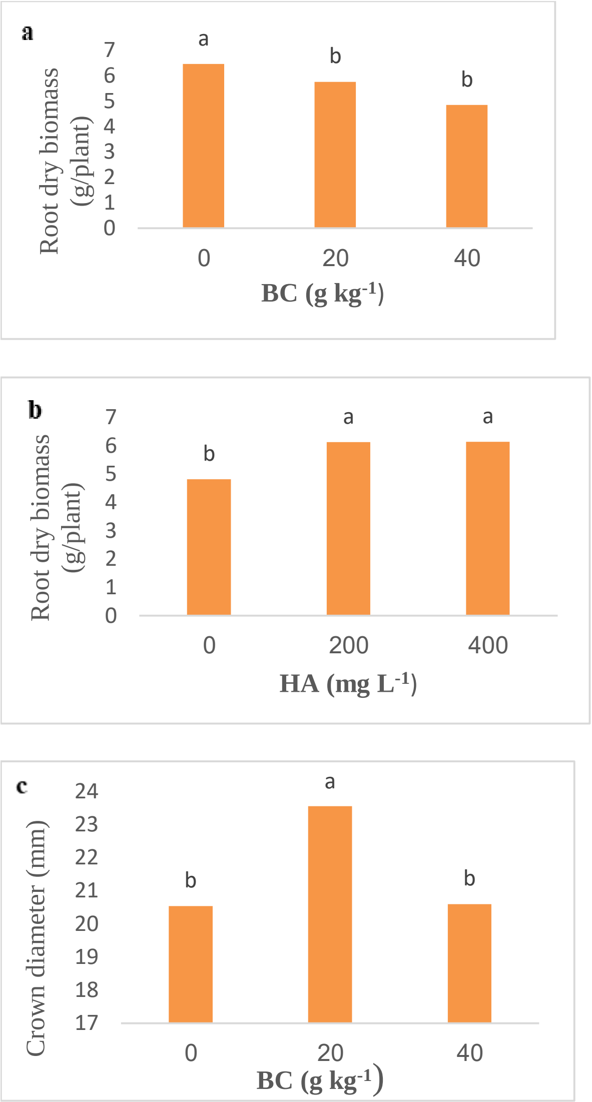

Fig. II. Effect of biochar on root dry biomass (a) and crown diameter (c) and effect of humic acid on root dry biomass (b) of blue fescue. Different letters are significant at $p < 0.05$ (LSD test).

Leaf Photosynthesis Pigments

The combination of biochar and humic acid increased chlorophyll a, chlorophyll b, and total chlorophyll content in blue fescue. The highest chlorophyll a and chlorophyll b were recorded at 40 g kg^{-1} BC with 200 mg L^{-1} HA and 40 g kg^{-1} BC with 400 mg L^{-1} HA, respectively (Fig. IV. a and Fig. IV. b), showing significant differences compared to the control. The highest total chlorophyll content was observed at 40 g kg^{-1} BC with 400 mg L^{-1} HA,

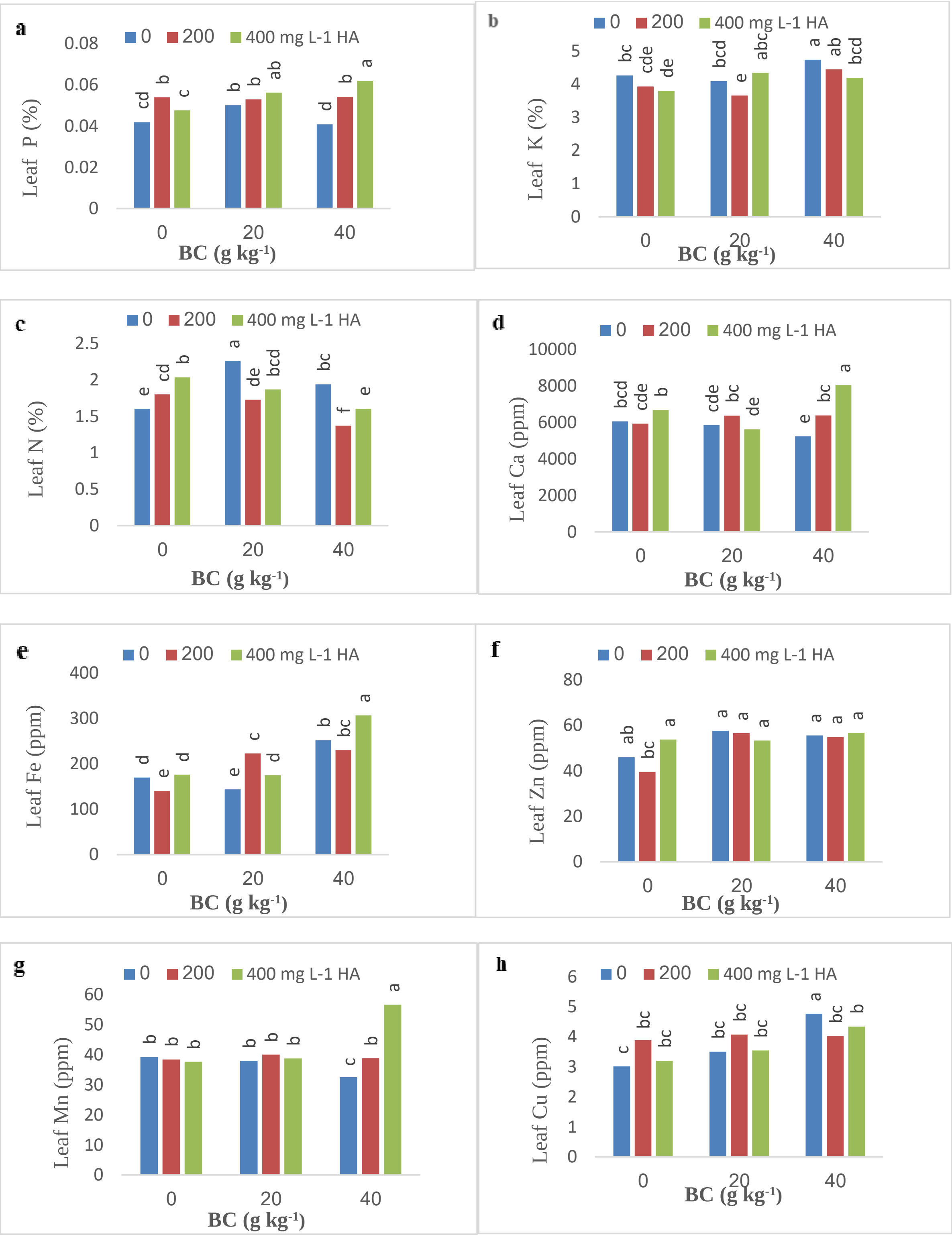

Fig. III. Effect of biochar and humic acid on leaf P (a), leaf K (b), leaf N (c), leaf Ca (d), leaf Fe (e), leaf Zn (f), leaf Mn (g), and leaf Cu (h) of blue fescue. Different letters are significant at $p < 0.05$ (LSD test).

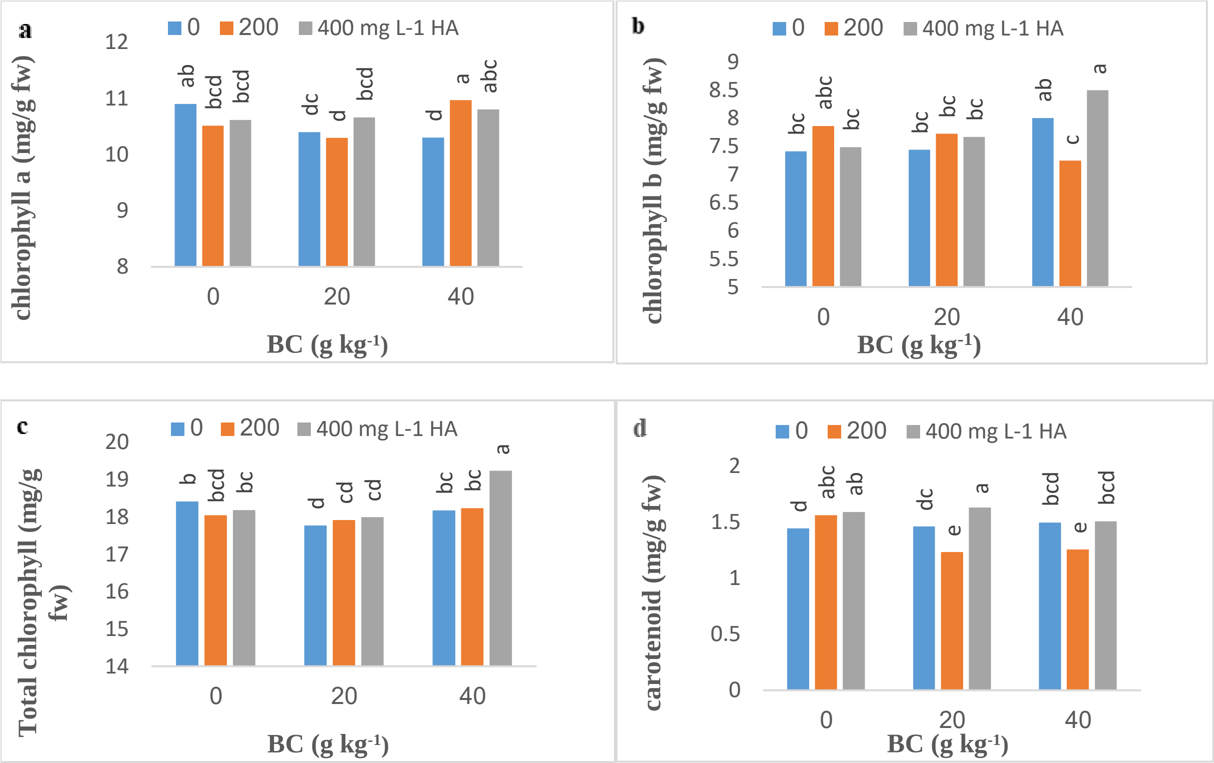

Fig. IV. Effect of biochar and humic acid on chlorophyll a (a), chlorophyll b (b), total chlorophyll (c), carotenoid (d), of blue fescue. Different letters are significant at $p < 0.05$ (LSD test).

which showed significant differences from other treatments (Fig. IV. c).

Discussion

Plant Growth Parameters

The plants Gerbera, Calathea, and Calendula show positive effects when treated with a combination of biochar (BC) and humic acid (HA) (Fornes and Belda, 2019; Karimi et al., 2020; Nikbakht et al., 2008).

The growth of crops, particularly rice and corn, can be enhanced through the use of biochar (Qin et al., 2016; Xiao et al., 2016). Conversely, a study by Chen et al. (2018) suggests that the influence of biochar on plant growth may not be enduring. Lavender plants grown with lower biochar in the substrate had better growth performance (Fascella et al., 2020). Biochar increases grape productivity, but its quality is not affected by biochar (Genesio et al., 2015). The characteristics of biochar, climate variations, application rates, soil types, environment, and crops contribute to the conflicting outcomes of biochar (Akhtar et al., 2014; Gul et al., 2015; Liu et al., 2016).

The relationship between biochar and plant growth parameters depends on the type and rate of biochar used in experiments. Different types of biochar affect nutrient content, as reported by Głąb et al. (2020). Moreover, the presence of biochar enhances water content in plants, leading to improved growth (Tayyab et al., 2018). Biochar can improve soil fertility, resulting in increased growth and yield (Zoghi et al., 2019). In grasslands, root biomass increased with the application of biochar (Głąb et al., 2020), which is consistent with our study.

The higher growth of roots with the use of humic acid could be due to greater allocation toward the roots in plants with humic acid addition, which is consistent with the results of Nikbakht et al. (2008). In tomato and basil, edaphic and foliar use of HA led to increases in quality, quantity, and early crop ripening (Abdipour et al., 2019; Eshghi et al., 2013; Yildirim, 2007). HA promotes aerial growth by increasing the uptake of minerals such as nitrogen, calcium, phosphorus, potassium, manganese, iron, zinc, and copper (Abdipour, Hosseinifarahi, and Najafian, 2019). Humic acids have beneficial effects on plant growth and development by direct and indirect effects on protein synthesis, alteration of enzyme activity, stimulation of photosynthesis and nutrient uptake, reduction of toxic elements, and increasing soil microbial flora (Abdipour, Hosseinifarahi, and Najafian, 2019).

Nutrient Concentration

Our findings are consistent with Karimi et al. (2020), Zhang et al. (2014), and Nikbakht et al. (2008), who reported that humic acid and biochar increase P, K, Ca, Fe, Mn, and Zn compared to the control. Biochar application led to a significant increase in N, P, and K content in Quercus castaneifolia compared to non-biochar soils (Zoghi et al., 2019). BC additions in medium culture increased plant nutrients in fly ash and eggplant (Belyaeva and Haynes, 2012; Kul, 2022). In *Centaurea cyanus* L., BC addition increased nutrient content, growth, and improved ornamental indices (Yang and Zhang, 2022).

Phosphorus (P) content in soil decreases with the use of biochar (Liang et al., 2014). On the other hand, Masto et al. (2013) stated that biochar promotes phosphorus mineralization in soil by increasing microbial biomass (Masto et al., 2013). Biochar may act as a P source in agriculture and increase plant growth (Manolikaki et al., 2016). Therefore, biochar increases the availability of P and K in soil and affects the nutrient content of plants (Chen et al., 2018). Amendment with biochar in a sand-based media (unfertilized) led to an increase in the release of nutrients from biochar to pore water (P and K), showing the potential for nutrient release from biochar. Also, increasing levels of Fe, K, Na, and P were observed in press water extracts with increasing rates of biochar (Kaudal et al., 2016). The increase in leaf Ca content in blue fescue cultivated with increasing biochar dosage was similar to that in apple rootstock and lavender species (Street et al., 2014). Realizing nutrient content from medium culture amended with biochar is the main reason for the higher content of nutrients in plants (Kaudal et al., 2016).

Humic acid (HA) may decrease the absorption of some elements, especially at higher concentrations (Nikbakht et al., 2008). It was demonstrated that the biomasses of pansies, lettuce, and basil were reduced as pH of the biochar increased. Similarly, the biomass of basil decreased when the electrical conductivity of biochar increased (Nobile, Denier, and Houben, 2020). HA influences the growth of the root system, causing lateral roots and root hairs to multiply and increasing the absorption and concentration of nutrients in the plant's aerial portions. However, HA promotes nutrition transfer in the aerial portion and increases the permeability of cell membranes (Abdipour, Hosseinifarahi, and Najafian, 2019; Karimi et al., 2020). In addition, HA has the potential to create complex mobile forms, chelate inaccessible nutrients, improve availability, and modify soil pH (Tahir et al., 2011).

Leaf Photosynthesis Pigments

Our findings about the effect of biochar on chlorophyll content are consistent with other studies (Hashem et al., 2019; Karimi et al., 2020). Applying biochar and humic acid could improve chlorophyll content by enhancing plant growth conditions in calendula (Karimi et al., 2020). It was reported that applying humic acid significantly increased chlorophyll content (Fascella et al., 2020; Karimi et al., 2020; Khorasaninejad, Alizadeh Ahmadabadi, and Hemmati, 2018).

Nutrient content may positively affect and increase chlorophyll content (i.e., P and K) in growing substrates (Netto et al., 2005). P and K are essential elements in the production of leaf photosynthesis pigments and plant photosynthetic activities. Deficiency of these elements leads to decreased photosynthesis due to reductions in chloroplast nutrient supply and issues in the Calvin cycle (Netto et al., 2005).

Leaf carotenoid content in Lavender increased in the substrate with biochar amendment (Fascella et al., 2020). In basil and tomato, the SPAD index was lower in BC-compost mixes due to lower leaf N content compared to the control (Gholizadeh et al., 2017). These results show that the type of BC can affect the availability of macro and micronutrients in plants and change other physiological properties.

Conclusion

Our findings in this study on blue fescue plants appear to suggest that pistachio biochar and humic acid have positive effects on growth parameters, photosynthesis pigments content and, nutrient uptake of blue fescue. The application of BC and HA, especially at a rate of 40 g kg−1 and 400 mg L−1, respectively, could improve most of the selected traits of this experiment. By combined application of BC and HA, their effect is much exhibited. Biochar and humic acid increase macro and micro nutrients of blue fescue and they recommend to use in growth substrate. Outcome of this research could be used for better growth of the blue fescue plant after transplanting and also growth in green space.

Acknowledgements

This work was supported by financial assistance from Ferdowsi University of Mashhad.
(Project code: 58526).

References

Abdipour, M., M. Hosseinifarahi and S. Najafian, 2019. 'Effects of humic acid and cow manure biochar (CMB) in culture medium on growth and mineral concentrations of basil plant'. *International Journal of Horticultural Science and Technology*, 6(1): 27-38.

Aboukila, E. F., I. N. Nassar, M. Rashad, M. Hafez and J. B. Norton, 2018. 'Reclamation of calcareous soil and improvement of squash growth using brewers' spent grain and compost'. *Journal of the Saudi Society of Agricultural Sciences*, 17(4): 390-397.

Agbna, G. H., S. Dongli, L. Zhipeng, N. A. Elshaikh, S. Guangcheng and L. C. Timm, 2017. 'Effects of deficit irrigation and biochar addition on the growth, yield, and quality of tomato'. *Scientia Horticulturae*, 222: 90-101.

Akhtar, S. S., G. Li, M. N. Andersen and F. Liu, 2014. 'Biochar enhances yield and quality of tomato under reduced irrigation'. *Agricultural Water Management*, 138: 37-44.

Ali, S., M. Rizwan, M. F. Qayyum, Y. S. Ok, M. Ibrahim, M. Riaz, M. S. Arif, F. Hafeez, M. I. Al-Wabel and A. N. Shahzad, 2017. 'Biochar soil amendment on alleviation of drought and salt stress in plants: a critical review'. *Environmental Science and Pollution Research*, 24: 12700-12712.

Belyaeva, O. and R. Haynes, 2012. 'Comparison of the effects of conventional organic amendments and biochar on the chemical, physical and microbial properties of coal fly ash as a plant growth medium'. *Environmental Earth Sciences*, 66: 1987-1997.

Bremner, J. and R. Hauck, 1982. 'Advances in methodology for research on nitrogen transformations in soils'. *Nitrogen in agricultural soils*, 22: 467-502.

Chen, G., J. Qiao, G. Zhao, H. Zhang, Y. Shen and W. Cheng, 2018. 'Rice-Straw Biochar Regulating Effect on *Chrysanthemum morifolium* Ramat. cv.'Hangbaiju''. *Agronomy Journal*, 110(5): 1996-2003.

Dispenza, V., C. De Pasquale, G. Fascella, M. M. Mammano and G. Alonzo, 2016. 'Use of biochar as peat substitute for growing substrates of Euphorbia× lomi potted plants'. *Spanish Journal of Agricultural Research*, 14(4): e0908-e0908.

Eshghi, S., M. Zare, B. Jamali, A. Gharaghani and M. H. Farahi, 2013. 'Vegetative and reproductive parameters of 'Selva'strawberry as influenced by Algaren, Drin and green hum foliar application'. *Ag Commun*, 1: 27-32.

Fascella, G., M. M. Mammano, F. D'Angiolillo, A. Pannico and Y. Rouphael, 2020. 'Coniferous wood biochar as substrate component of two containerized Lavender species: Effects on morpho-physiological traits and nutrients partitioning'. *Scientia Horticulturae*, 267: 109356.

Fornes, F. and R. M. Belda, 2019. 'Use of raw and acidified biochars as constituents of growth media for forest seedling production'. *New Forests,* 50(6): 1063-1086.

Gee, G. W. and J. W. Bauder, 1986. 'Particle-size analysis'. Methods of soil analysis: Part 1 Physical and mineralogical methods, 5: 383-411.

Genesio, L., F. Miglietta, S. Baronti and F. P. Vaccari, 2015. 'Biochar increases vineyard productivity without affecting grape quality: Results from a four years field experiment in Tuscany'. *Agriculture, Ecosystems & Environment,* 201: 20-25.

Gholizadeh, A., M. Saberioon, L. Borůvka, A. Wayayok and M. A. M. Soom, 2017. 'Leaf chlorophyll and nitrogen dynamics and their relationship to lowland rice yield for site-specific paddy management'. *Information Processing in Agriculture*, 4(4): 259-268.

Głąb, T., K. Gondek, M. Mierzwa-Hersztek and W. Szewczyk, 2020. 'Effects of straw and biochar amendments on grassland productivity and root morphology'. *Agronomy*, 10(11): 1794.

Gul, S., J. K. Whalen, B. W. Thomas, V. Sachdeva and H. Deng, 2015. 'Physico-chemical properties and microbial responses in biochar-amended soils: mechanisms and future directions'. *Agriculture, Ecosystems & Environment*, 206: 46-59.

Guo, Y., G. Niu, T. Starman, A. Volder and M. Gu, 2018. 'Poinsettia growth and development response to container root substrate with biochar'. *Horticulturae*, 4(1): 1.

Hashem, A., A. Kumar, A. M. Al-Dbass, A. A. Alqarawi, A.-B. F. Al-Arjani, G. Singh, M. Farooq and E. F. Abd_Allah, 2019. 'Arbuscular mycorrhizal fungi and biochar improves drought tolerance in chickpea'. *Saudi journal of biological sciences*, 26(3): 614-624.

Jarecka, K. and J. Sosnowski, 2021. 'The Effect of a Growth Stimulant Based on Iodine Nanoparticles on Festuca glauca'. *Journal of Ecological Engineering*, 22(11).

Karimi, E., M. Shirmardi, M. Dehestani Ardakani, J. Gholamnezhad and M. Zarebanadkouki, 2020. 'The effect of humic acid and biochar on growth and nutrients uptake of calendula (*Calendula officinalis* L.)'. *Communications in Soil Science and Plant Analysis*, 51(12): 1658-1669.

Kaudal, B. B., D. Chen, D. B. Madhavan, A. Downie and A. Weatherley, 2016. 'An examination of physical and chemical properties of urban biochar for use as growing media substrate'. *Biomass and Bioenergy*, 84: 49-58.

Kazemi, R., A. Ronaghi, J. Yasrebi, R. Ghasemi-Fasaei and M. Zarei, 2019. 'Effect of shrimp waste–derived biochar and arbuscular mycorrhizal fungus on yield, antioxidant enzymes, and chemical composition of corn under salinity stress'. *Journal of Soil Science and Plant Nutrition,* 19: 758-770.

Khorasaninejad, S., A. Alizadeh Ahmadabadi and K. Hemmati, 2018. 'The effect of humic acid on leaf morphophysiological and phytochemical properties of Echinacea purpurea L. under water deficit stress'. *Scientia Horticulturae*, 239: 314-323.

Kul, R., 2022. 'Integrated application of plant growth promoting rhizobacteria and biochar improves salt tolerance in eggplant seedlings'. *Turkish Journal of Agriculture and Forestry*, 46(5): 677-702.

Liang, Y., X. Cao, L. Zhao, X. Xu and W. Harris, 2014. 'Phosphorus release from dairy manure, the manure-derived biochar, and their amended soil: Effects of phosphorus nature and soil property'. *Journal of environmental quality*, 43(4): 1504-1509.

Lichtenthaler, H. K. 1987. '[34] Chlorophylls and carotenoids: pigments of photosynthetic biomembranes'. *In Methods in enzymology*. Elsevier, pp:

Liu, Y., H. Lu, S. Yang and Y. Wang, 2016. 'Impacts of biochar addition on rice yield and soil properties in a cold waterlogged paddy for two crop seasons'. *Field crops research*, 191: 161-167.

Major, J., M. Rondon, D. Molina, S. J. Riha and J. Lehmann, 2010. 'Maize yield and nutrition during 4 years after biochar application to a Colombian savanna oxisol'. *Plant and soil*, 333: 117-128.

Manolikaki, I. I., A. Mangolis and E. Diamadopoulos, 2016. 'The impact of biochars prepared from agricultural residues on phosphorus release and availability in two fertile soils'. *Journal of environmental management*, 181: 536-543.

Masto, R. E., M. A. Ansari, J. George, V. Selvi and L. Ram, 2013. 'Co-application of biochar and lignite fly ash on soil nutrients and biological parameters at different crop growth stages of Zea mays'. *Ecological Engineering*, 58: 314-322.

Murphy, J. and J. P. Riley, 1962. 'A modified single solution method for the determination of phosphate in natural waters'. *Analytica chimica acta*, 27: 31-36.

Netto, A. T., E. Campostrini, J. G. de Oliveira and R. E. Bressan-Smith, 2005. 'Photosynthetic pigments, nitrogen, chlorophyll a fluorescence and SPAD-502 readings in coffee leaves'. *Scientia Horticulturae,* 104(2): 199-209.

Nikbakht, A., M. Kafi, M. Babalar, Y. P. Xia, A. Luo and N.-a. Etemadi, 2008. 'Effect of humic acid on plant growth, nutrient uptake, and postharvest life of gerbera'. *Journal of Plant Nutrition*, 31(12): 2155-2167.

Nobile, C., J. Denier and D. Houben, 2020. 'Linking biochar properties to biomass of basil, lettuce and pansy cultivated in growing media'. Scientia *Horticulturae*, 261: 109001.

Qin, X., H. Wang, C. Liu, J. Li, Y. Wan, Q. Gao, F. Fan and Y. Liao, 2016. 'Long-term effect of biochar application on yield-scaled greenhouse gas emissions in a rice paddy cropping system: A four-year case study in south China'. *Science of the Total Environment*, 569: 1390-1401.

Sparks, D. L., A. L. Page, P. A. Helmke and R. H. Loeppert. 2020. Methods of soil analysis, part 3: Chemical methods: John Wiley & Sons.

Street, T. A., R. B. Doyle and D. C. Close, 2014. 'Biochar media addition impacts apple rootstock growth and nutrition'. *Hortscience*, 49(9): 1188-1193.

Tahir, M., M. Khurshid, M. Khan, M. Abbasi and M. Kazmi, 2011. 'Lignite-derived humic acid effect on growth of wheat plants in different soils'. *Pedosphere*, 21(1): 124-131.

Tayyab, M., W. Islam, F. Khalil, P. Ziqin, Z. Caifang, Y. Arafat, L. Hui, M. Rizwan, K. Ahmad and S. Waheed, 2018. 'Biochar: an efficient way to manage low water availability in plants'. *Applied Ecology & Environmental Research*, 16(3).

Xiao, Q., L.-X. Zhu, H.-P. Zhang, X.-Y. Li, Y.-F. Shen and S.-Q. Li, 2016. 'Soil amendment with biochar increases maize yields in a semi-arid region by improving soil quality and root growth'. *Crop and Pasture Science*, 67(5): 495-507.

Yang, W. and L. Zhang, 2022. 'Biochar and cow manure organic fertilizer amendments improve the quality of composted green waste as a growth medium for the ornamental plant Centaurea Cyanus L'. *Environmental Science and Pollution Research*, 29(30): 45474-45486.

Yildirim, E., 2007. 'Foliar and soil fertilization of humic acid affect productivity and quality of tomato'. *Acta Agriculturae Scandinavica Section B-Soil and Plant Science,* 57(2): 182-186.

Yuan, X., M. Gu, W. Teng, X. Yang and J. Wu, 2011. 'Growth of Calamagrostis brachytricha Steud. and Festuca glauca Lam. and estimated water savings under evapotranspiration-based deficit irrigation'. *The Journal of Horticultural Science and Biotechnology*, 86(6): 583-588.

Zhang, L., X.-y. Sun, Y. Tian and X.-q. Gong, 2014. 'Biochar and humic acid amendments improve the quality of composted green waste as a growth medium for the ornamental plant Calathea insignis'. *Scientia Horticulturae*, 176: 70-78.

Zoghi, Z., S. M. Hosseini, M. T. Kouchaksaraei, Y. Kooch and L. Guidi, 2019. 'The effect of biochar amendment on the growth, morphology and physiology of Quercus castaneifolia seedlings under water-deficit stress'. *European Journal of Forest Research*, 138: 967-979.