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Effects of Humic Acid and Cow Manure Biochar (CMB) in Culture Medium on Growth and Mineral Concentrations of Basil Plant

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Abstract
In sustainable agriculture, protection of the ecosystem and reduce chemical fertilizers are the main goals. Nowadays, sweet basil, one of the best general fragrant foliage, is considered as an industrial plant. In present study to compare different levels of cow manure biochar (CMB) and humic acid (HA) on vegetative traits and mineral absorption rate of green basil plants, an experiment was conducted based on a completely randomized block design with four replications in 2015. The treatments included different levels of CMB (0, 1%, 2%, and 3%) and HA (2.5, 5 and 7.5 g/L) in the culture medium. The traits that were analyzed were included: plant height, leaf number, leaf area, stem diameter, leaf chlorophyll concentration (LCC), fresh and dry weights of aerial part and root, concentration of potassium, nitrogen, calcium and phosphorus. Results showed that fresh and dry weights of shoot and root were increased by application of 3% CMB and 7.5 g/L HA in the culture medium. Theuse of CMB and HA significantly affected most of the studied traits except LCC, stem diameter, and leaf area. Concentration of N, P, K and Ca was considerably increased by application of 3% CMB and 7.5 g/L HA in the culture medium. The highest concentration of nutrients was detected in the plants treated with 3% CMB. In conclusion, the use of 3% CMB and HA at 7.5 g/L in the culture medium can be recommended to improve the qualitative and quantitative traits of basil plants.

Keywords: Cow manure biochar, Phosphorus, Potassium, Leaf chlorophyll concentration.

Introduction

Basil (*Ocimum basilicm* L.) is a medicinal plant, belonging to the Labiatae family, grown worldwide as one of the most aromatic, commonly-used and popular vegetables (Hosseini Farahi and Norouzinejad 2016). Types of basils have extensive medicinal and industrial applications (Naiji and Souri, 2018). The active ingredients of this plant are appetizing and can also be used for the treatment of bloating, enhancement of digestive tract, and treatment of some heart problems and enlarged spleen. Moreover, basil is used in traditional medicine as an expectorant, diuretic, anti-flatulence, stomach pain aid, and a stimulant (Kim et al., 2006).

Biochar is a charcoal prepared from plant biomass and agricultural waste. It is a stable, CO_2-rich compound and a highly porous, fine-grained material generated by heating the biomass (450-650 °C) under hypoxic/anoxic conditions, so-called

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pyrolysis (Kookana et al., 2011). This substance is resistant to decomposition, and its soil persistence is estimated by hundreds and even thousands of years depending on production conditions (Cheng et al., 2008).

Another feature of biochar is a high surface area (Liang et al., 2006). It can greatly influence nutrient dynamics and soil moisture (Glaser 2007). Biochar has a higher capacity to absorb cation than carbon in comparison with other organic materials of the soil, due to higher surface area, higher negative load and greater load density. As a result, biochar can help increase soil cationic exchange capacity (Liang et al., 2006). All of these traits make biochar an ideal complement for coarse-textured soils. The effects of agricultural biochar on crop yield and soil physical and chemical properties have been previously reported (Lehmann et al. 2003; Hejazizadeh et al., 2016; Zolfi Bavariani et al., 2016; Beheshti and Alikhani 2016; Ghorbani et al., 2016; Zhang et al., 2012).

Biochar has been reported to increase corn and rice yields, due to increased availability of nutrients and the improvement of soil physical properties (Zhang et al., 2012).

Humic compounds such as humic acid (HA) and fulvic acid contain a wide variety of organic and mineral compounds including amino acids, peptides, phenols, aldehydes, and nucleic acids bound to various cations found in nature. These substances indirectly improve soil cation exchangeability, buffering ability of substrate pH or nutrient solution. Furthermore, increased root absorption of macro-and microelements, improved soil structure, increased root volume, elevated water and air permeability of substrate are the other positive effects of these compounds.

It has been shown that humic compounds increase soil microbial flora and useful microorganisms, provide the root with certain special compounds such as nucleic acids, acetamides, provision of humic and fulvic acids as carriers of microelements and other growth factors, enhances absorption of nutrients, boosts plant resistance to drought, and increases soil fertility (Fallahi et al., 2006; Turkmen et al., 2004). Several studies have demonstrated the advantages of using HA in higher plants (Hosseini Farahi et al ,. 2015 ;Mohamadinea et al ,.2015 ;Dastyaran and Hosseini Farahi .2015 ;Fahimi et al ,. 2016) .

Yildirim (2007) showed that edaphic (soil) and foliar use of HA in tomatoes leads to increase in quality and quantity, and also early crop ripening. Atiyeh et al. (2002) reported that plant growth regulators (e.g., indolacetic acid, gibberellins, and cytokinins) present in HA had significant effects on plant growth and development. It has been shown that soil and foliar applications of HA increase vegetative growth traits, fruit weight and total yield, early ripening, and fruit quality in cucumber plants (Ozdamar ullu et al., 2011; Fahimi et al., 2016). Increased number of plant branches, leaf area average, total yield, mean fresh weight and phosphorus content were reported by the use of HA as foliar nutrition on bean plants (Zaky et al., 2006). El-Nemr et al. (2012) demonstrated that all morphological traits including plant height, number of plant leaves and stems, leaf fresh weight, yield and its components in cucumber presented positive and significant responses to the use of high HA concentrations compared with other treatments. Similar results were also obtained by Fahimi et al. (2016) on cucumber plants. An investigation on application of HA (100 mg/L) on grape plants revealed positive effects of HA on the size of grains and other qualitative parameters (Ferrara and Brunetti 2010). Improvement in growth parameters and yield of strawberry (var. Salva) have been reported as a result of HA treatment (Eshghi et al., 2013).

This study aimed to improve the qualitative and quantitative traits of basil plant using CMB and HA. Therefore, we investigated the effects of different CMB and HA levels on quantitative characteristics and mineral contents in green basil plants.

Materials and methods

Research location

This research was carried out on green potted basil plants at Gachsaran, Iran. The region is located at geographical coordinates of 30° and 18′ E and 51° and 13′ N from the Greenwich meridian at an altitude of 731 m.

Experimental design and treatments

The experiment was conducted based on a randomized complete block design with four replications. The treatments consisted of different CMB (0, 1%, 2%, and 3%) and HA (2.5, 5 and 7.5 g/L) levels with four replications and 10 plants were planted per pot. HA was used as a water-soluble powder (Humax 95 WSG, JH Biotech, USA) containing 80% HA, 15% folic acid, and 12% potassium. Physicochemical analysis of soil and biochar samples are presented in Table 1.

Biochar production

Dry cow manure was used to produce biochar at a pyrolysis temperature of 500 °C based on the production method of Lehmann and Rondon (Lehmann and Rondon 2006). The dry cow manure was placed in a capped ceramic chamber and heated in a muffle furnace (with indirect heating). Biochar was produced at 500 °C during 5 h. After the pyrolysis process, the biochar was pulverized and passed through a sieve with a size of 0.18 mm.

Preparation of plants

Culture media were first prepared depending on the treatments; after that green basil seeds were planted in the pots. Thinning of the plants began after germination and two-leaf stage, and 10 plants were cultivated per pot. Plants were irrigated regularly (every 3 days), and growing operations were carried out during the growth period. After 80 days, the data collection was carried out.

Vegetative traits

Plant height and root length (cm) were measured by a ruler. Stem diameter (mm) was determined by a digital caliper. The leaf green index was measured by hand-held model chlorophyll meter (SPAD 520-Minolta, Japan). Leaf area (mm^2) was estimated by a grid (squared) paper, and the number of leaves was determined by counting leaves per plant. The number of leaves was measured by counting the leaves per plant.

At the end of the experiment, the basil plants were carefully removed from the soil and after complete washing, the root length was measured with a ruler.

Fresh and dry weights of aerial and below-ground parts

At harvesting time, the plants were completely removed from the pots, roots were separated from the aerial part, and fresh weights of root and aerial organs were measured by a digital scale. Dry and fresh weights of aerial organs and root were calculated per treatment and average values were reported based on g per plant. After determining fresh weight, the plants were wrapped in aluminum foil and placed at 70 °C for 48 hours in an oven. After full drying, dry weights of the samples were measured and mean values were reported based on g per plant.

Measuring leaf mineral contents

For measuring leaf mineral contents, plant leaf samples were collected and transferred to the laboratory. The samples were washed with distilled water, incubated in an oven (70 °C) to reach a constant weight, and then powdered with an electric mill. After preparation of samples, nitrogen was measured by the Kjeldahl method, calcium with atomic absorption, phosphorus by colorimetric, and potassium by a flame photometer (Aboutalebi et al., 2007).

Statistical analysis

This experiment was conducted based on a completely randomized block design with seven treatments each with four replications. Data were analyzed using MSTATC software, and means were compared by

Duncan's multiple range tests at 1% probability level.

Results

Vegetative traits

The results showed that using CMB and HA significantly affected basil height. Table 2 shows that increasing CMB level in the medium and also feeding the plants with HA led to an increase in the height of basil plant when compared to the control. The highest plant height (12.88 cm) was observed in plants received 3% CMB and also those grown with 7.5 g/L of HA (12.43 cm) whereas the lowest height (8.97 cm) was recorded in untreated plants. Compared to control treatment, root length increased significantly by increasing the level of CMB and HA. Longest roots (10.25 cm) was observed in plants grown in a culture medium containing 3% CMB while those in control presented the shortest (6.5 cm) root length (Table 2). The results showed that CMB and HA applications had no significant effects on basil leaf area, stem diameter, and LCC values. However, the above-mentioned traits were improved insignificantly with increasing CMB in the culture media and feeding plants by HA compared to untreated plants (Table 2).

Table 1. Results of physicochemical analysis of soil and biochar sample.

Soil									
Cu ppm	Zn ppm	Mn ppm	Fe ppm	O.M %	C %	K Mg/kg	P Mg/kg	N %	pH
0.36	1.8	12.08	5.4	680	38.7	233	46	0.174	7.4
Biochar									
Cu ppm	Zn ppm	Mn ppm	Fe ppm	O.M %	C %	K %	P %	N %	pH
44	80	34	680	38.7	16	4	3.5	4.5	8.6

Table 2. Mean comparison for the effect of biochar and humic acid on vegetative characteristics of green basil plants

Treatment	Root length (cm)	Chlorophyll index (SPAD	Stem diameter (mm)	Leaf area (mm^2)	height of the stem (cm)
Control	6.5^{d}	21.91^{a}	1.66^{a}	6.25^{a}	9.97^{c}
Biochar 1%	8^{bcd}	23.15^{a}	1.72^{a}	6.66^{a}	11.2^{abc}
Biochar 2%	9.25^{abc}	24.31^{a}	1.95^{a}	7.42^{a}	12.13^{ab}
Biochar 3%	10.25^{ab}	24.4^{a}	2.21^{a}	7.5^{a}	12.87^{a}
Humic acid 2.5 g/l	7.5^{cd}	22.92^{a}	1.71^{a}	6.64^{a}	9.52^{bc}
Humic acid 5 g/l	8.75^{bcd}	23.47^{a}	1.86^{a}	7.42^{a}	11.55^{abc}
Humic acid 7.5 g/l	9.25^{abc}	24.14^{a}	2.05^{a}	7.5^{a}	12.43^{a}

Means within a column with the same superscript letters are not significantly different at $p < 0.01$ (DMRT test).

Number of leaves

The results of this experiment showed an increase in the number of leaves by the applications of CMB and HA compared to untreated plants. The highest number of leaves (79) was observed in the plants grown in 3% CMB medium, and untreated plants had the lowest number of leaves (17.75) (Fig. 1).

Fresh and dry weights of aerial part of plants

CMB and HA had significant influences on basil aerial parts. These two substances caused increases in both fresh and dry weights of aerial organs in comparison with untreated plants. The plants grown in the culture medium with 3% CMB and the control exhibited the highest and lowest weights of aerial parts, respectively (Fig. 2). Moreover, dry weight of aerial parts maximized in plants received 3% CMB in the culture medium while it minimized in untreated plants (Fig. 3).

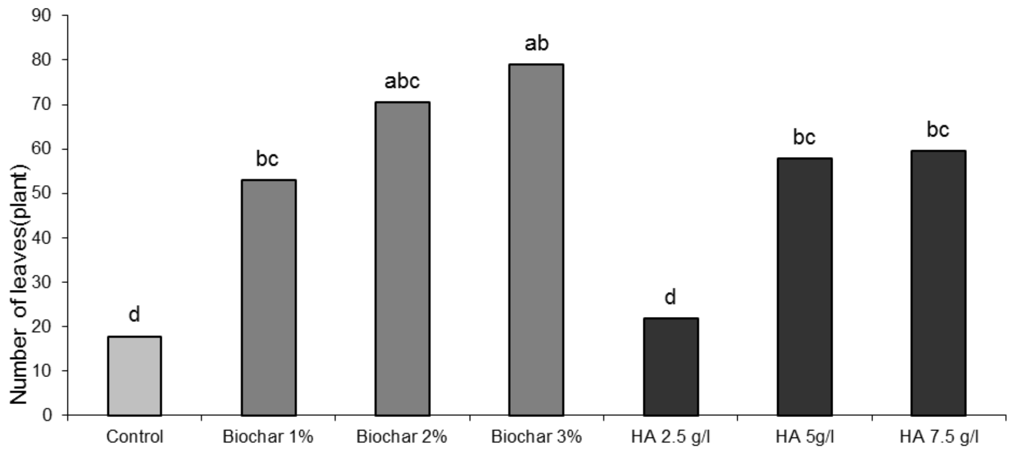

Fig. 1. Effect of cow manure biochar and humic acid (HA) on the number of leaves of green basil plants. Columns with the same letters are not significantly different at the 1% probability.

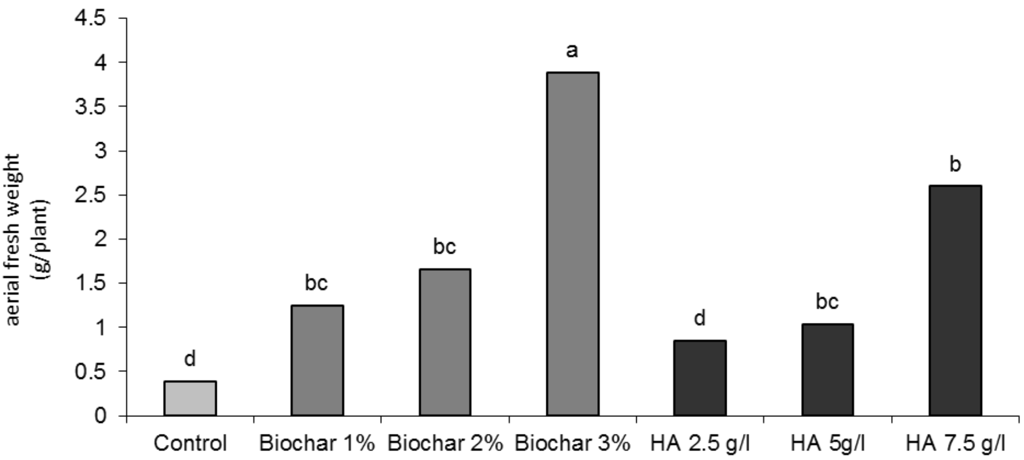

Fig. 2. Effect of cow manure biochar and humic acid (HA) on total fresh weight of green basil plants. Columns with the same letters are not significantly different at the 1% probability.

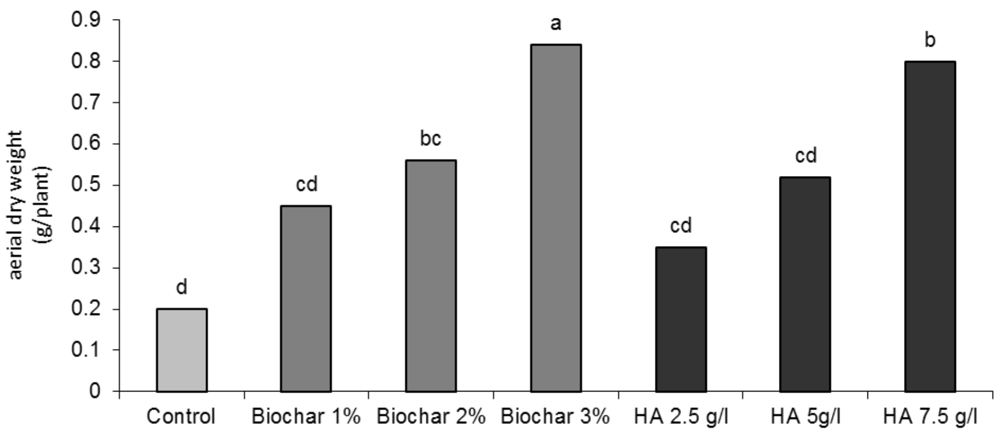

Fig. 3. Effects of cow manure biochar and humic acid (HA) on dry weight of aerial part of basil plants. Columns with the same letters are not significantly different at the 1% probability.

Fresh and dry weights of root

In this study, CMB and HA applications caused significant effects on basil root so that the fresh and dry weights of root were significantly increased in comparison with the control plants. The highest root weights were observed in plants planted in a culture medium containing 3% CMB and the lowest root weights was detected in control plants (Fig. 4). Furthermore, the highest and lowest root dry weights were observed in plants grown in the culture medium containing 3% CMB and the control, respectively (Fig. 5).

The highest root weights were observed in plants in a culture medium containing 3% CMB and the lowest root weight was measured in control plants.

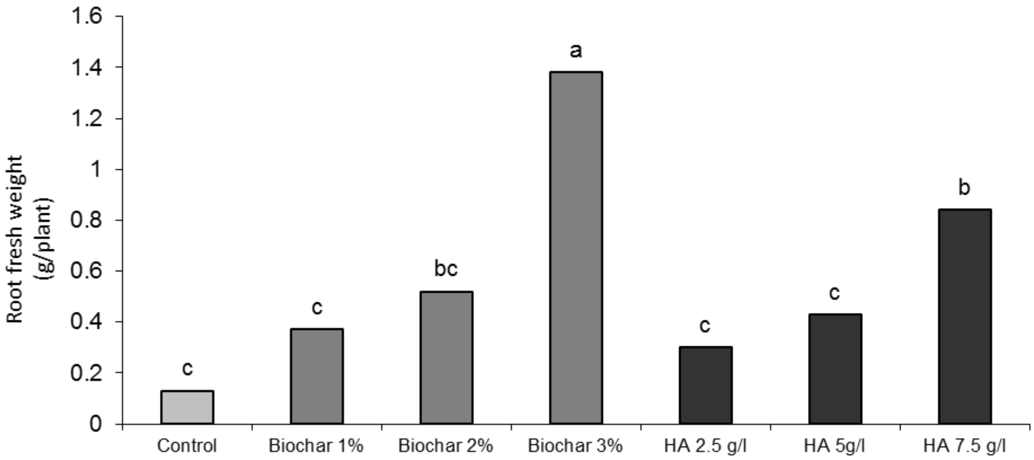

Fig. 4. Effects of cow manure biochar and humic acid (HA) on root fresh weight of green basil plants. Columns with the same letters are not significantly different at the 1% probability.

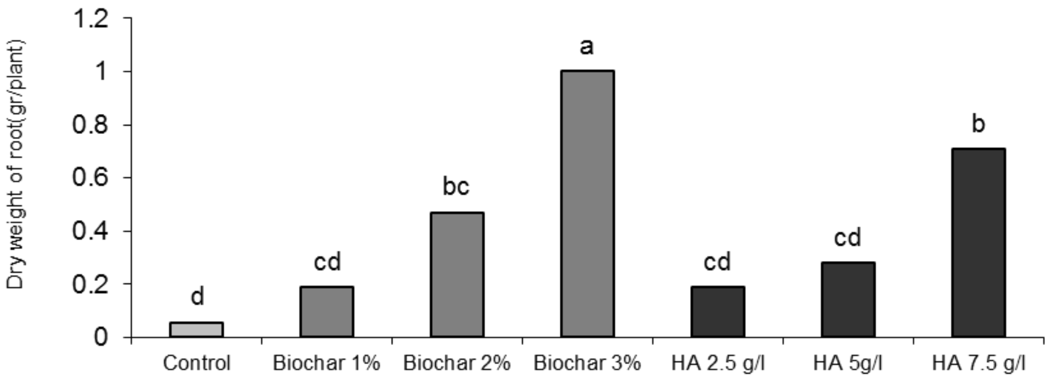

Fig. 5. Effects of cow manure biochar and humic acid (HA) on root dry weight of green basil plants. Columns with the same letters are not significantly different at the 1% probability.

Leaf mineral content

The results of this study showed that application of CMB and HA resulted in significant increase in the concentration of mineral elements including nitrogen, phosphorus, potassium, and calcium, in basil leaves compared to the control plants. Leaf nitrogen percentage was significantly increased with higher levels of CMB and HA in the culture medium when compared to the control plants. In addition, the highest leaf nitrogen concentration (2.66%) was detected in the leaves of plants that grown in the culture medium with 3% CMB and the lowest leaf nitrogen concentration was detected in the leaves of control plants (Fig. 6). Increasing CMB levels in the culture medium and also HA levels caused significant increases in phosphorus percentage of leaves compared to leaf phosphorus content of plants treated with lower levels of CMB and HA. The concentration of leaf phosphorus was highest in the leaves of plants treated by 3% CMB in the culture medium, and untreated plants contained the lowest phosphorus Level in their leaves (Fig. 7). Leaf potassium content increased significantly with higher levels of CMB and HA in comparison with leaf potassium content of plants treated with lower levels of CMB and HA. The concentration of leaf potassium was increased in the leaves of plants grown in the culture medium containing 3% CMB while the minimum level of leaf potassium was detected in the control plant leaves (Fig. 8). Increasing levels of CMB in the culture medium and HA concentrations led to a significant increase in leaf calcium percentage compared to other treatments. Highest and lowest calcium concentrations were detected in the leaves of plants treated with 3% CMB and untreated plants, respectively (Fig. 9).

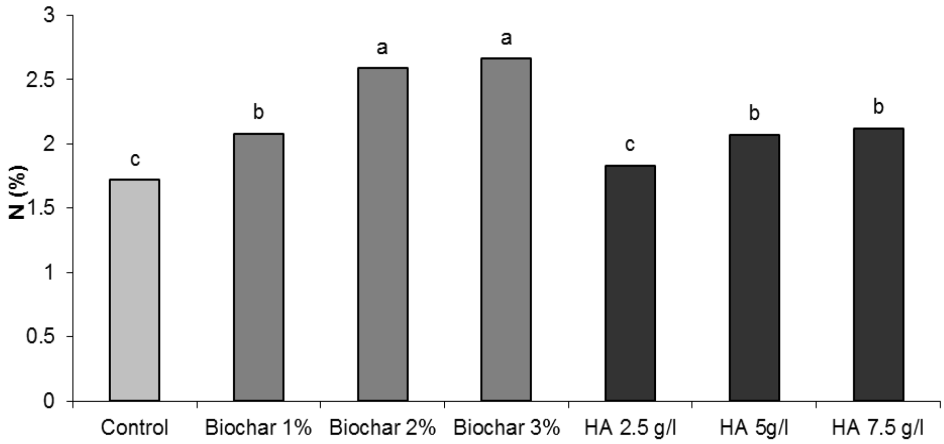

Fig. 6. Effects of cow manure biochar and humic acid (HA) on basil leaf nitrogen content. Columns with the same letters are not significantly different at the 1% probability.

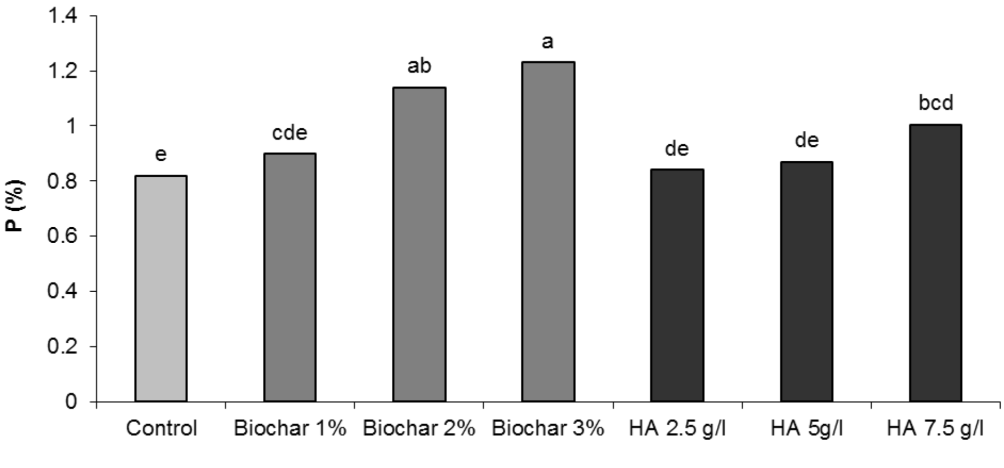

Fig. 7. Effects of cow manure biochar and humic acid (HA) on the content of leaf phosphorus. Columns with the same letters are not significantly different at the 1% probability.

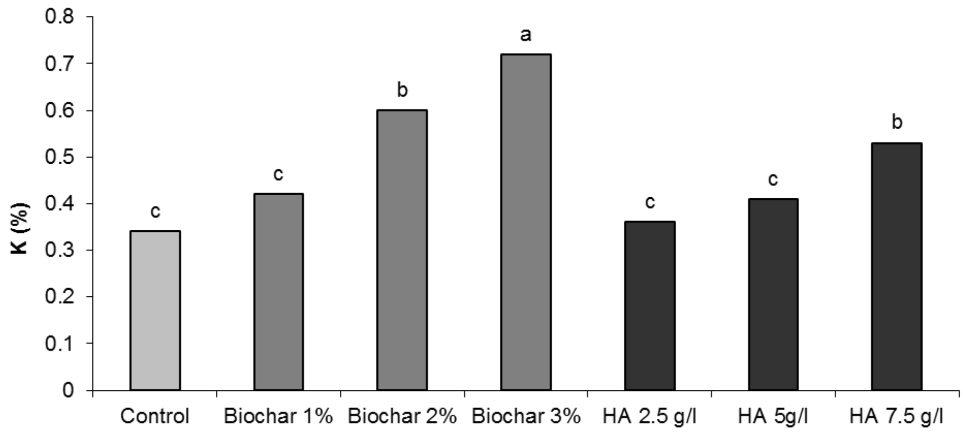

Fig. 8. Effects of cow manure biochar and humic acid (HA) on potassium content of basil leaf. Columns with the same letters are not significantly different at the 1% probability.

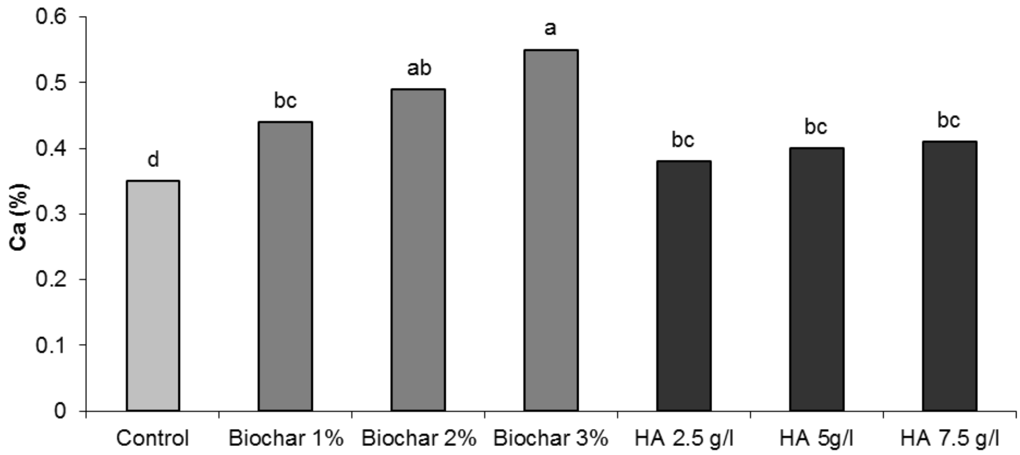

Fig. 9. Effects of biochar and humic acid (HA) on basil leaf calcium content. Columns with the same letters are not significantly different at the 1% probability.

Discussion

In the present study, application of CMB in the culture medium and also HA increased the vegetative characteristics including plant height, number of leaves, and fresh and dry weights of aerial parts and the root of basil plants, the positive effects of CMB was higher than that of HA. Some studies have shown that biochar application in the culture medium improves soil physical and chemical properties, preserves soil organic matter, increases nutrient productivity, and eventually improves crop yield (Vaccari et al., 2011). Aminifard et al. (2016) found that soil application of vermicompost can positively influence antioxidant compounds, fruit yield and quality of pepper. Increasing fresh and dry weights of aerial parts and root caused by biochar application can be attributed to increased soil nutrients availability and improved growth conditions, which resulting in improved yield. These positive effects can result from both direct and indirect effects of using biochar (e.g. nutrients in the biomass and improvement of soil physical, chemical and biological properties) (Major et al., 2010). Vaccari et al. (2011) reported improved wheat yield by biochar application (60 t/ha). Moreover, leaf area in soybean plants has been reported to increase with the use of biochar (Suppadit et al., 2012). Improvement of soil chemical properties and enhanced growth of beans and corn have been reported by use of poultry biochar (Inal et al., 2015). Positive effects of biochar application on wheat traits (stem length, tiller number, spike number, spike length, spikelets per spike and grain yield) were reported under both salt stress and non-stress conditions (Akhtar et al., 2014). Improved growth of lettuce has been also reported by application of poultry biochar and rice husk biochar (Gunes et al., 2014; Carter et al., 2013). It has been shown that application of CMB in sandy soil significantly increased the growth, yield, and uptake of nutrients in cornflower (Uzoma et al., 2011).

Our observations indicated that the use of CMB in the culture medium and also HA enhanced the absorption of nitrogen, phosphorus, potassium, and calcium into the basil leaves, but the inductive effects of CMB on nutrient accumulation in the leaf was higher than that of HA. Absorption of the above-mentioned minerals was maximal in plants that grown in a culture medium containing 3% CMB. Increasing amount of leaf nitrogen can result from soil fertilization due to the used fertilizers and better absorption of soil nutrients by the plant (Van Zwieten et al., 2010). They further recorded a similar effect of biochar on nitrogen uptake and found that biochar application to the soil increases nitrogen uptake by the plant. Major et al. (2010) presented evidence that the application of biochar significantly increases potassium uptake by corn grains and enhances rate of mineral uptake in maize cultivation. Similarly, increased phosphorus uptake in high ratios of applied biochar was also reported by Lehmann et al. (2003), which was attributed to a high level of phosphorus in cow manure available in the post-harvest, biochar-enriched soil.

In the present study, application of HA increased vegetative traits and mineral uptake compared to control plants, but the effect of biochar was more evident. HA has multiple effects, including promoting soil physical and chemical properties, increasing soil permeability and water holding capacity, complexation of metal ions, increasing cation exchange capacity (CEC), improving nutrients uptake, improvement of drought resistance of plants, and both direct and indirect effects on plant growth (Karakurt et al., 2008; Souri and Hatamian, 2019). Recently, Abootalebi Jahromi et al. (2016) indicated 3000 and 4000 mg/kg HA can be used to reduce the toxic effects of salinity and also to decrease the uptake of toxic elements such as sodium and chloride in Mexican lime seedlings (Abootalebi Jahromi et al., 2016).

HA increases nutrient uptake and concentration in aerial parts of plant through the expansion of root system, increasing cell membrane permeability, enhancing root nutrient uptake capacity, and finally improving nutrient transfer and uptake in the aerial part) Souri and Yaghoubi Sooraki, 2019). Fallahi et al. (2016) found that seed inoculation of plants by mycorrhiza and to some extent application of HA are two reliable strategies for Roselle production under deficit irrigation. Enhanced aerial growth results from increased uptake of minerals such as nitrogen, calcium, phosphorus, potassium, manganese, iron, zinc, and copper (Hayes and Clap 2001). The hypotheses that can be expressed for the effect of HA on plant growth parameters include: a combination of HA and mineral ions, catalysis of HA to enzymes in the plant, HA effects on respiration and photosynthesis, stimulation of nucleic acid metabolism, and hormonal activity of HA) Dastyaran and Hossein Farahi 2015; Hosseini Farahi et al,. 2013) .Recently; Barzegar et al. (2016) indicated that foliar application of putrescine and HA significantly increases fruit yield, vitamin C and proline contents, catalase and peroxidase activities (Barzegar et al,. 2016). They also found that HA at 300 mg l^{-1} and Put at 1.5 mM can improve growth, yield and quality of okra fruits (Barzegar et al,. 2016).

Some researchers reported that HA with a quasi-hormonal activity has considerable impacts on nutrient uptake, yield, and viability of calendula (Allahvirdizadeh and Nazari Deljou., 2014). Humic materials have beneficial effects on plant growth, development and production owing to direct and indirect effects on protein synthesis, simulation of photosynthesis, alteration of enzyme activity, solubilization and uptake of both micro- and macro-elements, reduction of active levels of toxic elements, increasing soil microbial flora, and quasi-hormonal activity in the plant (Sinha and Bhattacharyya 2011). An increase in the height of peppermint plant has been reported by HA application (Askari et al., 2012). Positive effects of HA organic fertilizers and compost have been reported on basil height, total aerial dry weight, and number of stems and also on uptake of iron, manganese, zinc and copper (Jamali et al., 2015).

Conclusion

Our findings demonstrate that application of CMB and HA to the culture medium resulted in quantitative and qualitative improvement of green basil plant due to improvement of root environment, increased activity of microorganisms, and better nutrient uptake. Therefore, based on the obtained result from present study application of 3% CMB and 7.5 g/L HA to the culture medium can improve the qualitative and quantitative characteristics of green basil plants. Therefore, their applications can be recommended for improving the yield and quality of herbal plants.

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