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Biochar application modified growth and physiological parameters of *Ocimum ciliatum* L. and reduced human risk assessment under cadmium stress

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ABSTRACT

Biochar (BC) is prepared from waste organic material that can improve soil health in the contaminated area. Soil pollution with cadmium (Cd) is one of the worldwide problems. The present study aimed to evaluate the BC influence on some morphophysiological and biochemical characteristics, also Cd concentration of *Ocimum ciliatum* L. leaves under Cd stress as well as human risk assessment. Therefore, a pot factorial arrangement based on a completely randomized design was done which included three levels of BC (non-BC, 1%, and 2% of the pot soil) and three Cd levels (0, 20, and 40 mg/kg soil) with three replications. The results of the present study indicated that BC application improved morphological traits, photosynthetic pigments, relative water content (RWC), and catalase (CAT) activity of *O. ciliatum* under Cd stress and reduced total soluble sugars, total phenol, antioxidant activity, proline content, electrolyte leakage (EL), soluble protein content, ascorbate peroxidase (APX), and guaiacol peroxidase (GPX) activities, and Cd concentration as well as target hazard quotient (THQ). In conclusion, based on the findings of this study, BC could be applied as an environmental friendly amendment in Cd-polluted soil to ameliorate the negative influences of Cd stress on *O. ciliatum* and reduces Cd levels and THQ in the plants due to the absorption properties of BC. This means that BC usage in contaminated soil helps to reduce pollutions and decreases the human risk assessment.

1. Introduction

Heavy metals contamination is one obstacle factor for plant development. Soil pollution by Cd is a serious problem because of using chemical fertilizers, pesticides, and sewage sludge (Yang et al., 2011). Among the trace elements, Cd enhancement in the soils becomes a serious problem worldwide (Rizwan et al., 2016a), so it is a danger to the health of the environment and humans. Among 20 metals with high toxicity, Cd is graded as the 7th one which is very dangerous for people and plants (Gill et al., 2012).

The average concentration of Cd in the soil was changed from neodymium to about 2.01 mg/kg in different regions of Iran (Amini et al., 2005; Solgi et al., 2012; Fattahi et al., 2019). Cd accumulates in the soils in different geochemical forms such as exchangeable and bounded. Their availabilities to plants are various include bioavailable (exchangeable and bounded to carbonates), potentially bioavailable (reducible and oxidizable fractions), and not available (residual fraction) (Alvarez et al., 2006; Obrador et al., 2007). Chemical forms and availability of Cd to plants are connected to the soil properties such as texture, CEC, pH, other cations concentration, and organic matter (Gallego et al., 2012; Umoren and Udousoro, 2009). Recently, Cd contamination in agricultural soils has become one of the most widespread and severe environmental and agricultural problems in Asian countries (Fan et al., 2010; Gallego et al., 2012; Mori et al., 2016). Enhancement in Cd concentration is being found in agricultural soils due to the chemical fertilizer application, organic manures, sewage irrigation, sludge usage, and atmospheric deposition (Inglezakis et al., 2014; Liang et al., 2017; Dharma-Wardana, 2018). The sources of Cd vary in different places. For instance, the potential risk of Cd accumulation in the farmland soil is more related to irrigation water compared with fertilizer and atmospheric deposition (Shiyu et al., 2020). Sewage sludge application to agricultural farmlands is very common worldwide because they are a low-cost source of plant nutrients and can improve soil properties. Nevertheless, sludge probably contains a high concentration of heavy metals such as Cd which have increased some effects regarding soil pollution. One study has concluded that greater metal mobility after sludge application (Moradi et al., 2005). Moreover,

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Nomenclature

ANOVA	Analysis of variance
APX	Ascorbate peroxidase
BC	Biochar
CAT	Catalase
Cd	Cadmium
CEC	Cation exchange capacity
DW	Dry weight
EDI	Estimated daily intake
EL	Electrolyte leakage
FC	Field capacity
FW	Fresh weight
GLM	General linear model
GPX	Guaiacol peroxidase
No.	Number
PTWI	Provisional tolerable weekly intake
ROS	Reactive oxygen species
RWC	Relative water content
THQ	Target hazard quotient
UV	Ultra violet

sewage sludge addition raises the inorganic part of the soil that can lead to an increase in the sorption affinity of soils for heavy metals (Moradi et al., 2005). In the arid region of central Iran, farmers extensively use sewage sludge as fertilizer. Due to the lack of controlling reducing pollutant concentrations in sewage sludge and the rate of sludge application on agricultural farmlands, high loads of heavy metals are introduced by sewage sludge application onto extremely farmed and excessively irrigated soils which may provide suitable conditions for heavy metal leaching. The long-term effect of Cd in the farmlands irrigated with Cd-enriched sewage sludge is important (Moradi et al., 2005). Thus, it seems that the most important Cd source in the studied region is irrigation water enriched sewage sludge that is polluted from materials from some factories and sewage. Depending on the sorption properties of the soil, the sludge adsorption capacity contributed by the inorganic fraction, and the sludge loading. Various effects of Cd on plants such as the nutrient absorption and distribution imbalance, growth inhibition, pigment decomposition, chlorosis, and finally death were observed (Yang et al., 2011; Gill et al., 2012). The root uptake and transfer Cd into the stem simply even though it is not necessary for the growth of some plant species. As a result, using Cd in the food chain possibly dangerous for people and other organisms (Rizwan et al., 2016b). The toxic influences of Cd on the health of plants and people were reported before in some researches (Nagajyoti et al., 2010; Rizwan et al., 2016b). In addition, some physiological disorders occur in the response to Cd stress. Cd increases the oxidative stress in plants by raising the ROS production in different plant parts. Moreover, the plant antioxidant defensive system disturbing which causes lipid peroxidation and oxidative stress (Gill and Tuteja, 2010). In addition, for protection against ROS, the plants improve non-enzymatic and enzymatic antioxidants like CAT, GPX, and APX (Vajpaee et al., 2000). Moreover, proline accumulation occurs under heavy metals stress. During stress in the plants, proline interfering in supporting the cell wall strength, inhibiting, and cleansing produced hydroxyls macromolecules degradation (Zarei et al., 2012).

There are various techniques to decrease the detrimental and toxic effects of heavy metals in plants. Organic additives such as BC have been revealed as highly effective and environment-friendly immobilizers to reduce Cd contamination (Hamid et al., 2020). BC is a carbonous pyrolyzed organic material used as a soil amendment in sustainable agriculture management (Lehmann and Joseph, 2015). The health of the soil and productivity of the crops are improved by BC due to its high carbon content (Lehmann et al., 2011; Ye et al., 2017; 2019a; 2019b; Yu et al., 2019; Kwon et al., 2020). It has a great surface area and its usage enhances soil quality, raises soil fertility and pH, improves water holding, and CEC which resulting in higher crop yields (Lehmann et al., 2011). Furthermore, the application of BC in the soils under plants cultivation decreases trace element toxicity and in the soil enhances metal immobilization (Younis et al., 2016; Abbas et al., 2017; Chang et al., 2019; Qiu et al., 2020; Khan et al., 2020a; Albert et al., 2020). Based on the findings of previous studies, BC was effective in promoting plant growth and reducing Cd accumulation (Qi, et al., 2020; Albert et al., 2020; Khan et al., 2020b). These influences of BC depend on the chemical and physical traits of BC such as the type of used materials, conditions of pyrolysis, the used method, and its rate, porosity, and specific surface area (Abbas et al., 2018). Furthermore, its application affects plant growth in different ways. It has various positive, detrimental, and neutral effects on the productivity of plants (Jeffery et al., 2011; Kwon et al., 2020). The influences of BC usage on crop growth have been studied before (Jeffery et al., 2011; Lehmann et al., 2011; Yu et al., 2019; Kwon et al., 2020), but the exact mechanistic of BC effects is not known completely. Under heavy element stress, there are several investigations regarding the positive influence of BC on the plants (Rehman and Ahmad, 2016; Zhang et al., 2016; Khan et al., 2020a). Therefore, BC application may provide a possible technology for green, environmental-friendly, and cost-effective remediation of Cd contaminated soil (Qi, et al., 2020; Albert et al., 2020; Khan et al., 2020b).

On the other hand, the heavy metal contaminated soils are potential threats to human health because of entering the toxic elements through the edible plants into the food chain and disrupt the health systems of humans. Such health risks can be decreased by suitable management of pollution sources, using remediation methods and plants pattern adjustment. Previously, BC application in contaminated soils decreased heavy metal concentration and alleviate associated health hazards (Khan et al., 2020a, 2020b).

BC indicated different effects on growth, biochemical, and physiological characteristics of the plants under Cd stress as schematic explanations have shown in Fig. 1.

Basil (*Ocimum* spp.) is one of the largest genera of Lamiaceae family which is native to Africa, Asia, and South America, especially the tropical and subtropical regions. The herbaceous parts of this aromatic plant have several attributes which are applied in food (as a vegetable, culinary), pharmaceuticals, and cosmetic industries (Makari and Kintzios, 2008; Moghaddam et al., 2011). As a traditional medicine, it has been used for curing some illnesses such as abdominal pains, constipation, worms, headaches, and kidney malfunctions (Makari and Kintzios, 2008). Between Iranian accessions of *Ocimum* spp., genetic diversity was done before. *Ocimum ciliatum* is one of the Iranian basil species which is grown at vegetable gardens and home in various areas of Iran. (Moghaddam et al., 2011).

Based on the literature, some reports about the effect of BC amendment on different traits of plants under Cd stress are available. But there was no research available on the effect of the BC on the studied traits of *O. ciliatum* under Cd stress until now. Since *O. ciliatum* is a valuable plant that is used as a culinary vegetable in Iran, consequently, the authors carried out this study to investigate the influences of BC on morphological, biochemical as well as physiological traits of *O. ciliatum*. On the other hand, one of the valuable parameters for estimating the human health risks of *O. ciliatum* that is consumed widely as an ordinary crop in the Iranian daily diet is the THQ. The low concentration of Cd can indicate no risk to human health. According to the literature review, until now there is no study to evaluate the role of BC in health risk reduction. Therefore, the authors evaluated the effect of BC on Cd content and human risk assessment of Cd in *O. ciliatum* for the first time.

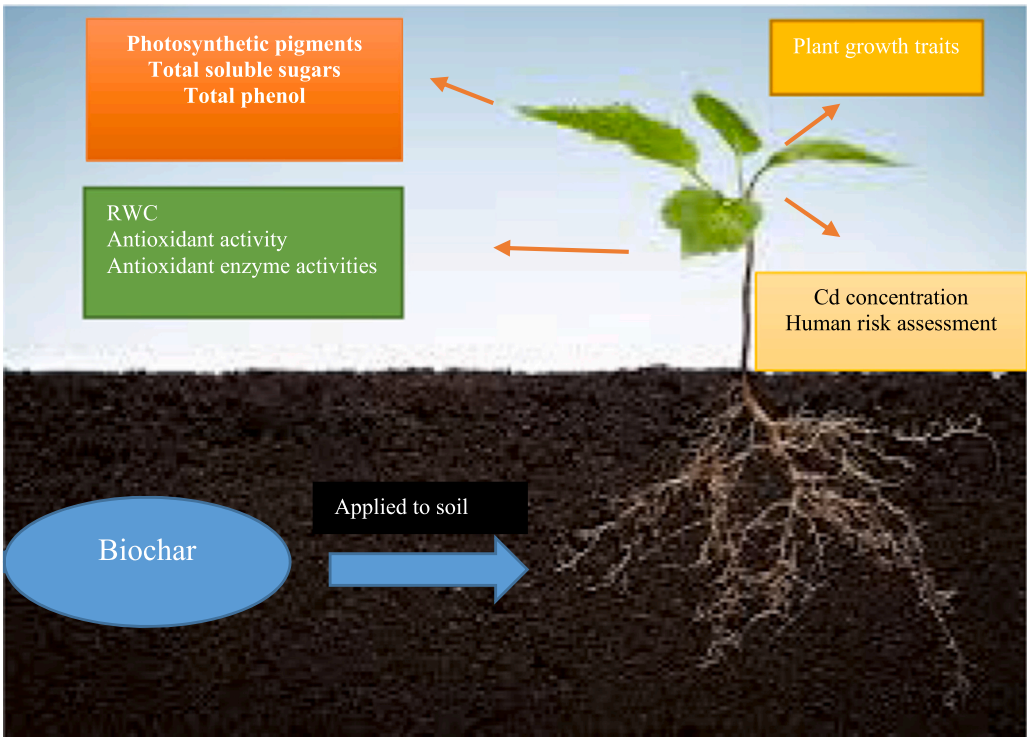

Fig. 1. Effects of BC on growth, biochemical and physiological traits of the plants under Cd stress.

2. Material and methods

2.1. Experimental design and growth conditions

A pot factorial experiment with three replications was done as a complete randomized design (CRD) in the greenhouse of Ferdowsi University of Mashhad, Iran. *O. ciliatum* L. seeds were prepared from Anbari Company, Mashhad, Iran. The sandy loam soil contained 73.4%, 18.3%, and 8.3% sand, silt, and clay, respectively. Soil pH, EC (dS/cm), CEC (cmol (+) kg^{-1}), and organic matters (%) values were 8.08, 3.09, 8, and 1.14, respectively. Other characteristics of the soil included: organic matter (1.14%), organic carbon (0.66%), N (0.06%), P (24.6 mg/kg), and K (202 mg/kg). The applied BC in this experiment was produced from air-dried wood residues of mulberry (*Morus alba* L.) under anaerobic conditions (530 °C for 14 h). After preparing BCs, they were powdered and sieved through a 1 and 2 mm sieve. The properties of produced BC include organic carbon 3.51%, particle size 1–2 mm; pH 9.7, bulk density 0.74 g cc^{-1}; EC 6.8 dS m^{-1}, cation exchange capacity (CEC) 5.2 cmol/kg, N, P, K, Na, and Cd amount were 0.97%, 0.43%, 1.23%, 0.19%, and Nd (neodymium), respectively. Each 12 kg plastic pot (30 cm × 40 cm) was filled with different amounts of desired doses of BC (non-BC, 1%, and 2% w/w of the pot soil). Before cultivating, different treatments of Cd were made ready. Cd treatments were prepared by spraying into the soil. Cadmium nitrate (Cd $(NO_3)_2$.4 H_2O) was used to obtain matrix concentrations of 0, 20, and 40 mg Cd kg^{-1} dry soil (mg/kg) (Sousa et al., 2014; Alamo-Nole and Su, 2017; Fattahi et al., 2019). The metal levels were selected by the Canadian Soil Quality Guidelines (1.4–22 mg Cd kg^{-1}) (Sousa et al., 2014). However, hazardous levels are reached for soil levels up to 30–40 mg/kg) (Pierzinsky et al., 2000).

Finally, the seeds were cultivated directly in each pot and at 4–6 leaf stages, only six vigorous seedlings remained in one pot. The plants were put in the greenhouse with 25 °C/18 °C day/night temperature, and 70–85% relative humidity. In addition, the soil water content holds on near to the field capacity (FC).

2.2. Plant assay and growth parameters

The plants were harvested at the full flowering stage and then the leaf samples were chosen from the 5th node to evaluate the morphological, biochemical, and physiological traits of the plants. The morphological traits of the plants included plant height, branchlet number, number of nodes, and number of flowering branches, inflorescence length, fresh and dry weight of the leaf, and the stem. Then the leaf and stem samples were oven-dried for 48 h at 72 °C and their dry weights were measured.

2.3. Extraction preparation

The fresh leaves (300 mg) were ground and extracted by 3 mL of methanol (99%). They were centrifuged at 4500 rpm for 10 min. Then they were filtered for separating insoluble portions and put for 24 h at 4 °C in the refrigerator.

2.4. Photosynthetic pigments content

After preparing the extraction, to measure chlorophylls and carotenoid content, the absorbance of extracts was calculated at 653, 666, and 470 nm by a spectrophotometer (Bio Quest C2502) (Wellburn, 1994).

2.5. Biochemical analysis

To determine total soluble sugar content, firstly anthrone reagent was prepared by solving anthrone (0.15 g) at 72% H_2SO_4 (100 mL). After that, the mixture reaction consisted of arranged anthrone (3 mL), and methanolic extract (100 μL) was prepared, mixed well, and put for 10 min in 100 °C bath. Finally, at room temperature, they were cooled and measured at 630 nm by a spectrophotometer (Mc Cready et al., 1950).

For determining total phenols, methanolic extract (100 μL), 50% Folin–Ciocalteu (200 μL), distilled water (100 μL) was mixed, after

3 min 7.5% sodium carbonate was added, for 1 h put in darkness, and the absorbance was reported at 765 nm (Singleton and Rosi, 1965).

To evaluate antioxidant activity, the mixture reaction included 1 mL of 2,2-Diphenylpicrylhydrazyl radical (DPPH) 500 mM and leaf extractions. They shake strongly at room temperature and put for 30 min in the darkness. Then the absorbance was recorded at 517 nm (Hanato et al., 1988). For calculating the percentage of inhibition of free radical DPPH (IP%), the following formula was used:

$$IP\% = \frac{Ablank - Asample}{Ablank} \times 100$$

A_{blank}: control reaction; A_{sample}: the presence of plant extract.

For measuring Leaf proline content, the leave samples (0.1 g) were extracted with 10 mL sulfosalicylic acid (3.3% w/v)), then filtered, acidic ninhydrin (2 mL), and 2 mL glacial acetic acid were added to the extract (2 mL). After that, for 60 min the tubes were heated in a water bath (100 °C), put at room temperature (25 °C), and for 30 min in an ice bath. Also, 4 mL toluene was used to extract the mixture. The absorbance of the fraction was recorded at 520 nm by a spectrophotometer (Bates et al., 1973).

2.6. Relative water content (RWC)

To evaluate the RWC of growing basil plants at the flowering stage, a part of the leaf samples were crashed and packed in a plastic bag for weighing the FW. After that, the samples were oven-dried for 48 h at 72 °C to evaluate the DW. Finally, the RWC of the plant was measured as the moisture loss through drying separated into sections by the fresh weight (Ben Taârit et al., 2012). To measure RWC% the following formula was used:

$$RWC\% = \frac{FW - DW}{TW - DW} \times 100$$

2.7. Electrolyte leakage (EL)

For measuring initial electrolyte leakage (EL1), the sample of the leaf was washed, sliced to about 0.1 cm, put in the filled tested tubes with 10 mL deionized water and put at room temperature for 24 h. After that for interrupting the tissues and releasing all electrolytes, tubes were covered and autoclaved at 121 °C for 15 min. At room temperature, the solution cooled, and then interrupted tissues (EL2) were evaluated (Lutts et al., 2004). To calculate electrolyte leakage percentage (EL%) the following formula was used:

$$EL(\%) = (EL1/EL2) \times 100$$

2.8. Protein extraction

The fresh leave samples (300 mg) were selected for preparing enzyme extraction, then they were homogenized in 50 mM potassium phosphate buffer (3 mL, pH=7.5) included EDTA (1 mM), polyvinylpyrrolidone (PVP) (1% (w/v)) as reaction mixture. Soluble protein content was measured by monitoring the absorbance at 595 nm (Bradford, 1976).

2.9. Assays of antioxidant enzyme activities and lipid peroxidation

To determine CAT activity, the reaction mixture contained 2.8 mL of 50 μM phosphate buffer (pH=7), 0.1 mL of enzyme extract, and 15 mM H_2O_2 (0.03 mL) mixed rapidly. Changes in absorbance at 240 nm in an interval of 30 s for 1 min were reported on a UV visible spectrophotometer (Beers and Sizer, 1952).

Guaiacol peroxidase (GPX) activity was measured by Jia et al. (2013) method. The reaction mixture included 50 μM potassium-phosphate buffer (pH=7), 1% guaiacol and 0.3% H_2O_2. The oxidation of guaiacol to tetraguaiacol was described on the basis of the raise in absorbency at 470 nm for 3 min

To evaluate APX activity, the reaction of the mixture included 50 μM potassium phosphate buffer (pH=7), 0.015 mM of H_2O_2, 0.5 mM of ascorbic acid, and 50 μL enzyme extract. The reaction was begun by adding H_2O_2, and ascorbate oxidation was calculated at 290 nm for 1 min (Jebara et al., 2005).

The level of malondialdehyde (MDA) content (as an end product of lipid peroxidation) was assessed. To determine MDA content of the leaves, the reaction mixture included enzyme extract (150 μL), 1 mL TCA solution (20% (w/v) in addition to thiobarbituric acid (TBA) (0.5% w/v). The mixture was mixed completely and incubated at 95 °C for 30 min. Then cooled at room temperature and centrifuged for 5 min (6149 g). The absorbance was reported at 532 nm and 600 nm by a spectrophotometer (Stewart and Bewley, 1980).

2.10. Cadmium measurement in plants

The sample of the leaf was ground and digested in HNO_3-$HClO_4$ (3:1, v/v) by holding them overnight. Then, adding more HNO_3 (5.0 mL) and putting the leaf samples on a hot plate, and digesting them until obtaining a clear solution. Finally, the Cd concentration was evaluated with an atomic absorption spectrophotometer. Atomic absorption spectroscopy (AAS) is an analytical technique that measures the concentrations of elements. It makes use of the absorption of light by these elements to measure their concentration. Atomic-absorption spectroscopy quantifies the absorption of ground-state atoms in the gaseous state. The absorption spectrum of Cd in its gaseous atomic state comprises a series of extremely narrow lines appearing by the electronic transitions of outermost electrons. In the case of Cd, most of these transitions are owned by visible and absorb ultraviolet (UV) regions. For the analysis, the sample of Cd is changed into atomic vapor and after that, the atoms absorb UV or visible light (at a selected wavelength) which is specific for Cd is measured and makes transitions to higher electronic energy levels. The analysis concentration of Cd is determined from the amount of absorption. Concentration measurements are determined from a working curve after calibrating the instrument with standards of known concentration (Paul et al., 2017; Abbas et al., 2018).

2.11. Human risk assessment of cadmium

The ability of health risk of cadmium in the plants can be evaluated by the similar methods used for estimating carcinogenic or no carcinogenic risks. For calculating no cancer risk assessment, the THQ (Target Hazard Quotient) was introduced by the US Environmental Protection Agency (Zhu et al., 2013).

The non-cancer risk description methodology of THQ was utilized to conclude the possibility of health risks in some of the contaminate (Storelli, 2008). The THQ for the user of possibly pollutant plants was calculated by contrasting the PTWI (Provisional Tolerable Weekly Intake) of each measured element in the plants. THQ was calculated on the basis of the following formula (Qian et al., 2010).

$$THQ = (EDI \times 7)/PTWI$$

EDI (Estimated Daily Intake) was measured based on the following equation (Nejabat et al., 2017):

$$EDI = (EF \times ED \times FIR \times C)/(W_{AB} \times TA)$$

EF (exposure frequency): 365 days/year.

ED (Exposure Duration): 70 years, equivalent to the average lifetime.

FIR (Food Ingestion Rate): 0.68 g/person/day.

C (Concentration): the heavy metal concentration in medicinal plants (mg/g).

W_{AB}: the average body weight (70 kg).

TA (Time Average): 365 days/year ×ED (the average exposure time for non-carcinogens).

PTWIs for Cd showed by Joint FAO/WHO Expert Committee on Food Additives (JECFA) which are 7 μg Cd/kg bw/week (Commission CA, 2011).

2.12. Statistical analysis

The finding data of the tested traits were subjected to General Linear Model (GLM) statistical analysis by Minitab 17 software. The combined effects of BC and Cd stress were analyzed by two-way analysis of variance (ANOVA), with a probability defined at $P < 0.05$. The means were compared with Bonferroni as a post hoc test and data are reported as mean values ± Standard Error (SE). For drawing figures, Microsoft Excel software was used.

3. Results

3.1. Growth characteristics

The findings of ANOVA in this study showed that the interaction effect of BC application under Cd stress was significant ($P < 0.05$) in the case of growth characteristics. Although the growth traits of the plants included plant height, branches number, number of nodes, number of inflorescences, inflorescence length, fresh and dry weight of leaf and stem reduced under Cd stress, BC usage increased these growth characteristics (Table 1). The highest plant height (51.8 cm), branchlet number (17.1), number of nodes (7.5), number of flowering branches (5.8), inflorescence length (22.7 cm), fresh (14 g/plant) and dry (4.2 g/plant) weight of the leaf, fresh (10 g/plant) and dry (4.8 g/plant) weight of the stem were observed at the highest BC usage (2% w/w) without Cd stress (Table 1).

At the highest level of Cd (40 mg/kg), using 2% w/w BC increased plant height, branches number, number of nodes, number of inflorescences, inflorescence length, fresh and dry weight of the leaf, fresh and dry weight of the stem by 39.8%, 27.64%, 21%, 31.25%, 5.5%, 38.35%, 140%, 37.31%, and 42.85%, respectively compared with the treatment 40 mg/kg Cd without using BC (Table 1).

3.2. Biochemical and physiological characteristics

According to the findings of the ANOVA in this study, the interaction effect of BC application under Cd stress was significant ($P < 0.05$) in the case of biochemical and physiological characteristics.

3.3. Photosynthetic pigments

The findings of this experiment indicated that a significant decrease in chlorophylls and carotenoids concentrations in the leaves with raising Cd concentration (Table 2). The lowest chlorophyll a (2.1 mg g^{-1}FW), b (1.8 mg g^{-1}FW), total chlorophyll (6.2 mg g^{-1}FW), and carotenoid (0.9 mg g^{-1}FW) were observed at the highest concentration of Cd level (40 mg/kg) with no BC usage (Table 2). Moreover, the application of BC under Cd stress causes chlorophylls and carotenoids content to increase. At the highest Cd level (40 mg/kg), using 2% w/w BC improved chlorophyll a, b, total, and carotenoids by 57.14%, 50%, 20.96%, and 55.55% compared with the same treatments with no BC usage (Table 2).

3.4. Biochemical analysis

The finding of the present study indicated that by increasing Cd concentration, the total soluble sugars of plants increased significantly (Table 3). The highest total soluble sugars (108.8 mg g^{-1}FW) was obtained at the 40 mg/kg Cd concentration without using BC (Table 3). Total soluble sugars decreased by BC usage. At 40 mg/kg Cd concentration, using 2% w/w BC decreased total soluble sugars by 25.6% in comparison without using BC (Table 3).

According to the results of this research, with increasing Cd level, total phenols content increased significantly (Table 3). The highest total phenols (32.3 mg g^{-1}FW) was reported at the highest level of Cd (40 mg/kg) without using BC (Table 3). BC usage decreased total phenols content at 40 mg/kg Cd concentration about 39.4% compare with the treatment without using 2% w/w BC (Table 3).

The findings of this research indicated that with increasing Cd level, the antioxidant activity of plants increased (Table 3). The highest antioxidant activity (82.3%) was reported at the highest level of Cd (40 mg/kg) without BC usage (Table 3). BC application reduced antioxidant activity and at 40 mg/kg Cd concentration using 2% w/w BC decreased antioxidant activity by about 31.3% (Table 3).

In the present study, proline accumulation as a result of Cd stress was observed (Table 3). The highest proline content (0.0016 μMpro g DW^{-1}) was seen at the 40 mg/kg Cd concentration without using BC (Table 3) and BC usage decreased proline content significantly.

Table 1
Cd and BC levels influences on morphological traits of *O. ciliatum*.

Cadmium concentration (mg/kg)	Biochar (%of the pot soil)	Plant height (cm)	Branchlet no.	No. of nodes	No. of inflorescence	Inflorescence length (cm)	Fresh weight of the leaf (g/plant)	Dry weight of the leaf (g/plant)	Fresh weight of the stem (g/plant)	Dry weight of the stem (g/plant)
0	0	46.6 ± 0.4 BCE[a]	16.0 ± 0.4 ab	7.8 ± 0.3 a	3.8 ± 0.3 cd	18.6 ± 0.3 b	10.1 ± 0.1 b	2.6 ± 0.03 b	7.2 ± 0.2 b	3.0 ± 0.1 de
20	0	43.2 ± 0.7c	14.3 ± 0.2 b	6.9 ± 0.1 b-d	3.2 ± 0.2 cd	17.5 ± 0.1 b	8.8 ± 0.4 c	2.4 ± 0.1 b	6.7 ± 0.3 b	2.8 ± 0.1 e
40	0	32.6 ± 0.1 d	12.3 ± 0.6c	6.2 ± 0.0 d	3.2 ± 0.6 d	16.4 ± 0.6 b	7.3 ± 0.03 d	1.5 ± 0.2 c	6.7 ± 0.1 b	2.8 ± 0.03 e
0	1	47.3 ± 1.1 b	16.2 ± 0.2 a	7.5 ± 0.3 ab	5.5 ± 0.3 ab	19.4 ± 0.1 ab	13.4 ± 0.3 a	4.1 ± 0.04 a	9.4 ± 0.2 a	4.8 ± 0.0 a
20	1	45.3 ± 0.4 BCE	16.0 ± 0.4 ab	7.2 ± 0.1 a–e	4.4 ± 0.2 a–d	17.6 ± 0.9 b	10.2 ± 0.3 b	2.9 ± 0.1 b	7.1 ± 0.4 b	3.5 ± 0.1 cd
40	1	42.9 ± 0.2c	16.0 ± 0.4 ab	6.5 ± 0.1 cd	4.0 ± 0.2 b–d	17.3 ± 1.2 b	9.2 ± 0.2 BCE	2.6 ± 0.01 b	7.0 ± 0.1 b	2.9 ± 0.2 de
0	2	51.8 ± 0.6 a	17.1 ± 0.1 a	7.5 ± 0.1 ab	5.8 ± 0.4 a	22.7 ± 0.6 a	14.0 ± 0.2 a	4.2 ± 0.1 a	10.0 ± 0.2 a	4.8 ± 0.2 a
20	2	45.3 ± 1.4 BCE	15.7 ± 0.2 ab	7.5 ± 0.3 ab	5.2 ± 0.1 a–c	18.8 ± 0.4 b	12.8 ± 0.1 a	4.0 ± 0.2 a	9.8 ± 0.3 a	4.4 ± 0.2 ab
40	2	45.6 ± 0.8 BCE	15.7 ± 0.12 ab	7.5 ± 0.3 ab	4.2 ± 0.1 b–d	17.3 ± 0.4 b	10.1 ± 0.4 b	3.6 ± 0.1 a	9.2 ± 0.1 a	4.0 ± 0.0 BCE

[a] Data are mean ± SE (Standard Error). Different letters in a column indicated the statistically significant differences at 5% level probability of the means based on the Bonferroni analysis.

Table 2
Cd and BC levels influences on photosynthetic pigments content of *O. ciliatum*.

Cadmium concentration (mg/kg)	Biochar (%of the pot soil)	Chlorophyll a (mg g^{-1}FW)	Chlorophyll b (mg g^{-1}FW)	Total chlorophyll (mg g^{-1}FW)	Carotenoid (mg g^{-1}FW)
0	0	5.9 ± 0.9 BCE[a]	4.8 ± 0.7 b	13.6 ± 1.0 b	1.6 ± 0.1 ab
20	0	4.0 ± 0.4 b–d	4.9 ± 0.3 b	12.1 ± 0.8 BCE	0.9 ± 0.1c
40	0	2.1 ± 0.5 d	1.8 ± 0.4 d	6.2 ± 0.5 d	0.9 ± 0.0 c
0	1	7.0 ± 0.8 b	4.8 ± 0.2 b	19.9 ± 0.3 a	1.6 ± 0.2 ab
20	1	5.4 ± 0.5 b–d	3.6 ± 0.2 b–d	9.4 ± 0.8 b–d	1.5 ± 0.0 a–c
40	1	3.3 ± 0.3 cd	2.9 ± 0.2 b–d	7.5 ± 0.2 cd	1.3 ± 0.2 BCE
0	2	13.9 ± 1.4 a	8.0 ± 0.4 a	22.8 ± 1.8 a	1.9 ± 0.0 a
20	2	13.5 ± 0.2 a	4.0 ± 0.4 BCE	11.3 ± 0.9 BCE	1.8 ± 0.1 ab
40	2	3.3 ± 0.03 b–d	2.7 ± 0.0 cd	7.5 ± 0.4 cd	1.4 ± 0.1 a–c

[a] Data are mean ± SE (Standard Error). Different letters in a column indicated the statistically significant differences at 5% level probability of the means based on the Bonferroni analysis.

Table 3
Cd and BC levels influences on biochemical characteristics of *O. ciliatum*.

Cadmium concentration (mg/kg)	Biochar (%of the pot soil)	Total soluble sugar (mg.g^{-1}FW)	Total phenols (mg g^{-1}FW)	Antioxidant activity (%)	Proline content (μMpro/gDW)	RWC (%)	EL (%)
0	0	44.02 ± 2.29 e[a]	18.78 ± 0.27 b–d	62.14 ± 1.25 b–d	0.0007 ± 0.00005 cd	63.09 ± 1.00 b	25.23 ± 2.58 b–d
20	0	92.87 ± 0.32 ab	22.88 ± 1.35 b	70.76 ± 2.85 b	0.0011 ± 0.00008 a–c	55.87 ± 0.63c	26.71 ± 0.83 b
40	0	108.77 ± 4.37 a	32.29 ± 1.69 a	82.35 ± 0.69 a	0.0016 ± 0.00023 a	56.77 ± 1.51c	36.70 ± 0.83 a
0	1	38.93 ± 1.96 ef	15.37 ± 0.15 d	51.56 ± 0.03 de	0.0006 ± 0.00002 d	66.25 ± 0.003 b	19.89 ± 1.81 cd
20	1	65.08 ± 3.45 cd	20.22 ± 0.00 c	65.87 ± 2.82 BCE	0.0009 ± 0.00002 b–d	64.12 ± 1.22 b	26.20 ± 0.21 BCE
40	1	104.07 ± 5.22 a	29.38 ± 0.00 a	66.88 ± 3.87 BCE	0.0012 ± 0.00010 ab	56.26 ± 0.003c	36.22 ± 0.71 a
0	2	21.63 ± 1.89 f	15.11 ± 0.24 d	50.25 ± 0.78 e	0.0001 ± 0.000008 e	78.65 ± 0.76 a	19.21 ± 0.24 d
20	2	57.59 ± 6.20 de	16.12 ± 0.70 cd	55.91 ± 0.81c–e	0.0008 ± 0.00006 b–d	73.15 ± 0.91 a	25.45 ± 0.81 b–d
40	2	80.88 ± 5.16 BCE	19.58 ± 0.24 BCE	56.59 ± 2.53c–e	0.0009 ± 0.00005 b–d	65.56 ± 1.76 b	28.42 ± 0.46 b

Different letters in a column indicated the statistically significant differences at 5% level probability of the means based on the Bonferroni analysis.
[a] Data are mean ± SE (Standard Error).

3.5. RWC

The results of this experiment indicated that RWC decreased by raising Cd levels. The lowest RWC (55.9%) was obtained at 40 mg/kg Cd without BC usage treatment, although this treatment had no significant variation between 20 mg/kg Cd level without using BC and using 1% BC at 40 mg/kg Cd (Table 3). BC application particularly at 2% w/w significantly increased RWC. At 40 mg/kg Cd and 2% w/w BC usage, RWC increased about 15.48% in comparison with the treatment without using BC (Table 3).

3.6. EL

The findings of this study indicated that EL increased by increasing Cd levels. The highest EL (36.7%) was obtained at 40 mg/kg Cd without BC usage treatment, although using 1% w/w BC at 40 mg/Kg Cd treatment had no significant difference with this treatment (Table 3). Application of BC particularly at 2% w/w decreased EL significantly. At 40 mg/kg Cd concentration, 2% w/w BC usage decreased EL about 22.56% in comparison with the treatment without using BC (Table 3).

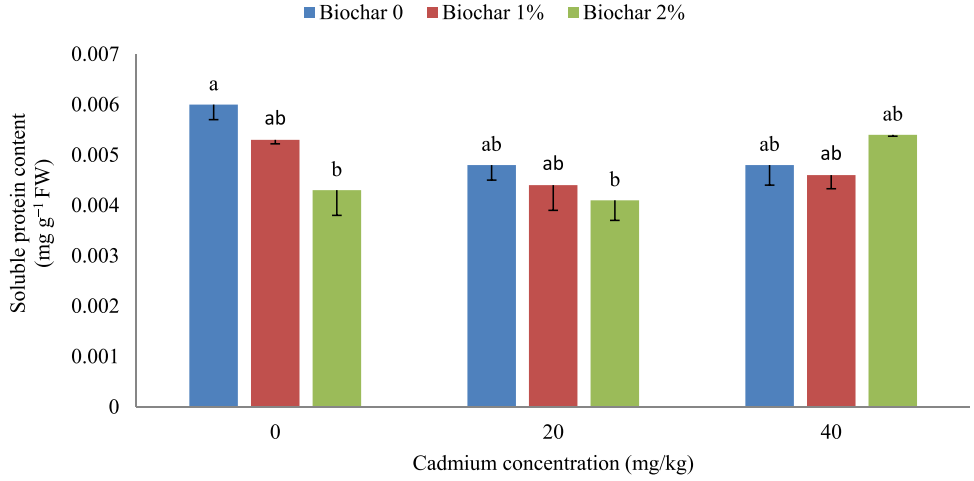

Fig. 2. Cd and BC levels influences on protein content of *O. ciliatum*. Bars with the similar letters are not significantly different at ($P < 0.05$) based on Bonferroni analysis.

3.7. Soluble protein content

The findings of the present experiment showed that soluble protein content decreased by increasing Cd levels. The highest content of soluble protein (0.006 mg g^{-1} FW) was obtained at control treatment (Fig. 2). BC usage decreased soluble protein content, although no significant statistical difference between the treatments was reported (Fig. 2).

3.8. Antioxidant enzyme activities and lipid peroxidation

In the present experiment, by increasing Cd concentrations, CAT activity was reduced (Fig. 3a). The lowest CAT activity (0.023 unit mg^{-1} protein) was observed at the highest Cd level (40 mg/kg) without using BC (Fig. 3a). But BC usage increased CAT activity under Cd stress (Fig. 3a). Moreover, APX activity significantly increased by increasing Cd concentrations (Fig. 3b). The highest APX activity (2.56 unit mg^{-1} protein) was obtained at the highest Cd level (40 mg/kg) without using BC (Fig. 3b). Application of BC under Cd stress decreased APX activity and the lowest APX activity (1.25 unit mg^{-1} protein) reported at the treatment of 40 mg/kg Cd by using 2% w/w BC usage (Fig. 3b). In addition, by raising Cd concentrations GPX activity significantly increased (Fig. 3c). The highest GPX activity (0.035 unit mg^{-1} proteins) was reported at the treatment of 40 mg/kg Cd without using BC (Fig. 3c). Under Cd stress, the application of BC decreased GPX activity and the lowest GPX activity (0.016 unit mg^{-1} protein) was observed at the treatment of 40 mg/kg Cd by 2% w/w BC application (Fig. 3c).

Lipid peroxide production determines oxidative damage to plant cell membranes. Therefore, an increase in MDA content is regarded as evidence of cell membrane injury. The plants grown under the highest Cd concentration (40 mg/kg) showed considerable injury to their cells. The highest MDA content (82.15 mmol g^{-1}FW) was observed under the highest Cd concentration (40 mg/kg) with no BC usage. Application of BC (particularly 2% w/w) compensated the injury caused by Cd stress in all Cd concentrations (Fig. 3d).

3.9. Leaf Cd concentration

In the present study, Cd concentration in the leaves increased by raising Cd levels (Fig. 4). The highest Cd concentration was obtained at 40 mg/kg Cd treatment without using BC (Fig. 4). BC usage in the soil decreased the Cd content of the plants in comparison with the growing plants in the soil with no BC usage. BC usage (2% w/w) at 40 mg/kg Cd concentration reduced Cd content by 32.68% in comparison with the

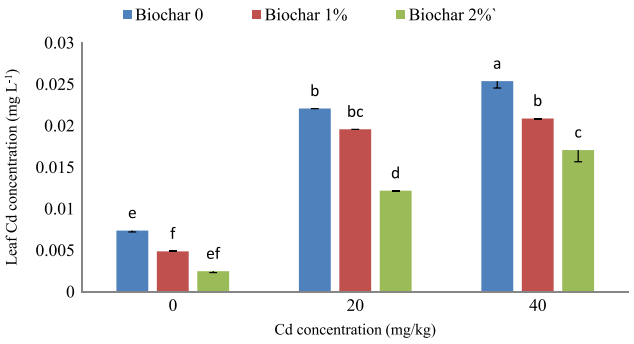

Fig. 4. Cd and BC levels influences on a) Cd concentration in the leaves of *O. ciliatum*. Bars with the similar letters are not significantly different at ($P < 0.05$) based on Bonferroni analysis.

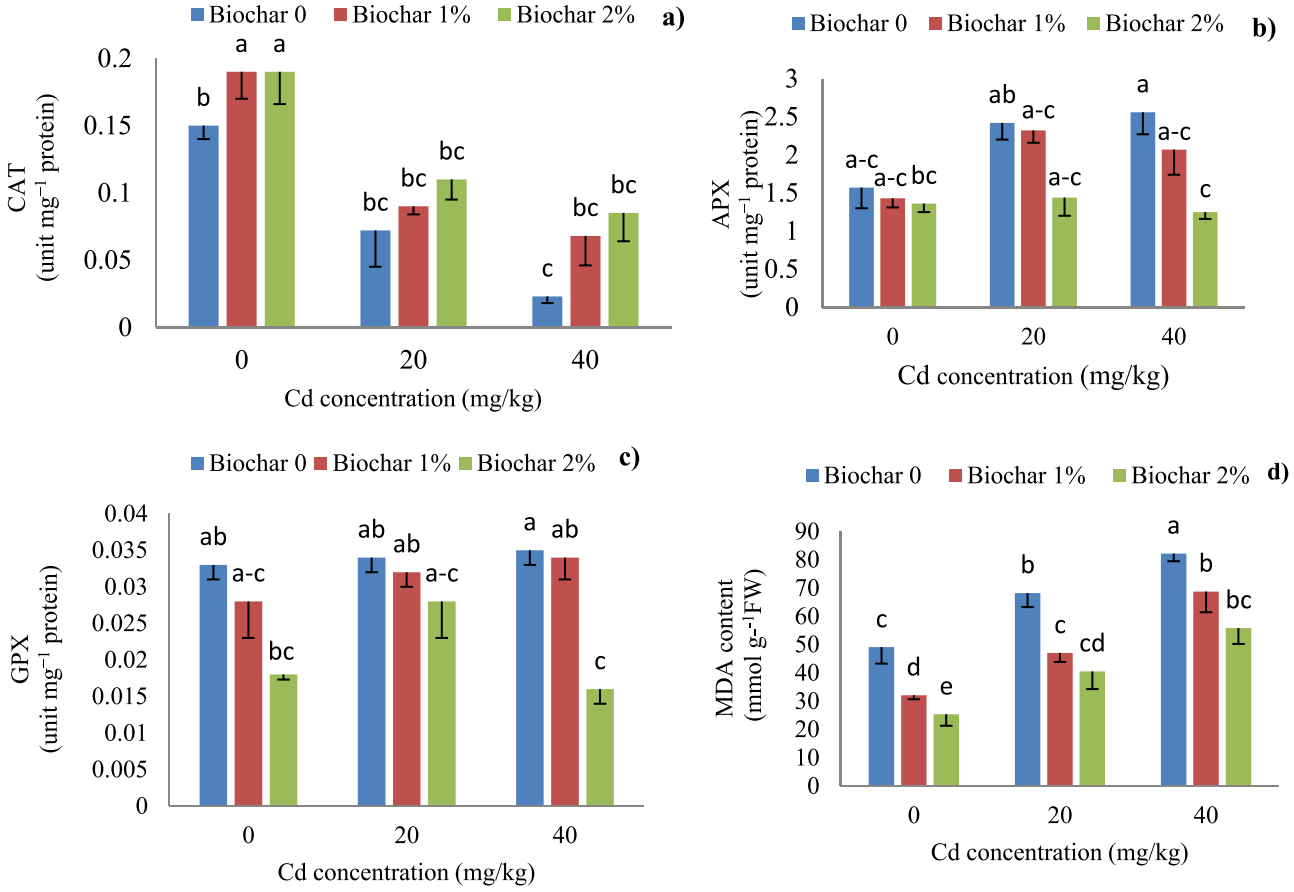

Fig. 3. Cd and BC levels influences on (a) CAT activity, (b) APX activity, c) GPX activity, and d) MDA content of *O. ciliatum*. Bars with the similar letters are not significantly different at ($P < 0.05$) based on Bonferroni analysis.

treatment without using BC (Fig. 4).

3.10. Human risk assessment of cadmium

The THQ for estimation of Cd risk displayed by consumption of *O. ciliatum* is presented in Fig. 5. Based on the results, using BC decreased THQ in all Cd treatments. At the highest Cd concentration (40 mg/kg), BC usage (2% w/w) decreased THQ by 30.38% compared with the treatment with no BC.

4. Discussion

One toxic element for plants is Cd which can reduce the growth, yield, and plant quality (Rizwan et al., 2016a; Xie et al., 2019). The reduction in growth factors connected to Cd concentration in the soil may be as a result of a reduction in cell wall constituents, mitosis inhibition, Golgi apparatus damage, and changes in the metabolism of polysaccharides (Sai Kachout et al., 2010). Cell meiosis and growth of plants reduce by heavy metals because of decreasing the photosynthesis and exchange of the products of photosynthesis. Cd accumulation leads to Mg, Ca, and Fe lack, and therefore chlorophyll synthesis is interrupted. So, photosynthetic volume decrease causes to stop plant growth by injuring chloroplast thylakoid membranes and stopping the Calvin cycle enzymes activities like stomatal cells, electron transfer chain, and Rubisco destruction (Jianpeng et al., 2010). Moreover, they diminish the toxic influences of free radicals by their role in detoxification. On the other hand, in Cd stress, the amount of Mg^{2+} and phosphorus absorption, which are necessary for chlorophyll and photosynthetic pigment synthesis, is decreased (Jianpeng et al., 2010). Also, reduction in photosynthetic pigments under Cd stress may be a result of oxidative injuries and due to the inhibitory effects on photosynthetic pigment synthesis. Under metal stress, some enzyme activities of the Calvin cycle, which decrease photosynthesis, and the growth of the plants are stopped (Nagajyoti et al., 2010). In addition, photosynthetic pigments reduce under Cd stress may be due to the reduction in biomass under stressful conditions. These pigments have defensive influences versus oxidative stress and therefore they are demolished under metal stress (Vajpaee et al., 2000).

From the other point of view, the most observable evidence for amendment-induced stresses in the plants is their physiological responses of them. Under stress conditions, increasing in total soluble sugars maintain various membranes and different cellular structures from harmful influences of stress (Rohani et al., 2019). In the previous report, the elevation of soluble sugars under Cd stress has been described (Rohani et al., 2019) which is in line with the results of the present report. By increasing soluble sugars under stress conditions, the carbohydrate storage of plants keeps at the desired level for supporting basal

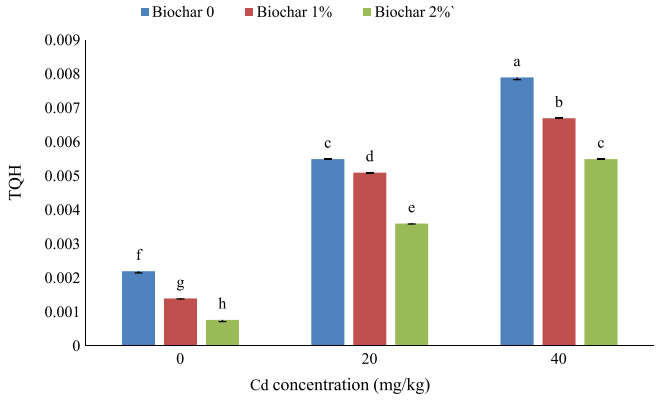

Fig. 5. Cd and BC levels influences on TQH in the leaves of *O. ciliatum*. Bars with the similar letters are not significantly different at ($P < 0.05$) based on Bonferroni analysis.

metabolism (Rohani et al., 2019). Furthermore, enhancing sugar concentration in plants connected to water reduction in cells is a significant agent in reducing plant growth.

In addition, phenolic composition with antioxidant activity is found in several plant species (Gill et al., 2012). Also, phenolic compounds have an influential use in absorbing and counteracting free radicals, alleviating singlet oxygen, and breaking up peroxide (Ahmad et al., 2015). Stress conditions result in controlling the involved enzyme activities in polyphenols synthesis and cause effective scavenging of toxic radicals and increasing plant growth (Wada et al., 2014). Phenolic compounds in plants may reveal various necessities to prevent stresses (Rohani et al., 2019). Besides, phenols can change oxidative stress and enhance the stability of membrane causes of supporting plant growth (Ahmad et al., 2015). On the other side, under stressful conditions, phenol synthesis improves to preserve the cellular structure in opposition to oxidative stress (Rohani et al., 2019). The accumulation of total phenols under Cd stress suggested that the phenolic compounds may be stress-induced in basil. The findings of this study are like the results of the previous researches on *Cannabis sativa* (Ahmad et al., 2015) and *Pistacia vera* (Rohani et al. 2019) that indicated enhancing total phenolic content under stress conditions. Under stress conditions in plants, excessive ROS is produced that highly reactive and toxic and damages carbohydrates and proteins. Thus plants enhance active oxygen scavenging systems to avoid these damages (Krishnaiah et al., 2010). Therefore, increasing in phenolic content of basil probably has a protective strategy due to the potential of relieving ROS. The potential of herbal medicines in reducing ROS-induced tissue damage is estimated in several experiments increasingly (Krishnaiah et al., 2010). The correlation between polyphenols and antioxidant activity in plants was studied before (Rainha et al., 2011) that in accordance with the findings of this report. The antioxidant potential of plants is very important for applying in pharmaceutical and food industries to provide natural products. Moreover, the antioxidant molecules can control ROS levels, and in the detoxification of free radicals; they have important functions (Rohani et al., 2019). In addition, antioxidants can prevent lipid, protein, and other vital molecules oxidation in cells as well as inhibit the initiation or reproduction of oxidizing chain responses (Krishnaiah et al., 2010).

Besides, one of the influential osmolytes associated with conserving tissue water is proline. Osmolyte accumulation like proline assists the plants to maintain cell water ability in soil solution (Rohani et al., 2019). Under Cd stress, accumulation of proline was described in several plants included *Lycopersicon esculentum* (Hayat et al., 2011), *Brassica juncea* (Irfan et al., 2014), *Pistacia vera* (Rohani et al., 2019) which confirm the findings of this experiment. Under Cd stress condition, proline increases because its synthesis from glutamic acid is stimulated, its export through the phloem is decreased, its oxidation during stress is inhibited and the process of protein synthesis collapsed (Hayat et al., 2011).

Additionally, one of the most activated proteins in plants is a soluble protein which is included enzymes and metabolism modulators that sensitive to stresses (Yin et al., 2016). The findings of this experiment on the Cd effect on reducing proteins in conformity with the findings of the previous study in *Pistacia vera* (Rohani et al., 2019). Under heavy metal stress, produced oxygen-free radicals can control the enzymes via attacking antioxidant enzymes and oxidative damages. Cd stress brings about oxidative stress and reduces antioxidant enzyme activities in different species of plants (Nagajyoti et al., 2010). The membrane structure modifies under Cd stress and changes in lipid peroxidation, and therefore an alternation happens in antioxidant enzyme activities (Sai Kachout et al., 2010). To getting rid of oxidative stress, antioxidant enzymes like APX and CAT are important factors in plants, also under stress conditions one or more such enzymes increase. Then CAT activity decreases and H^+ accumulation increases which in turn causes controlling CAT enzymes (Vajpaee et al., 2000). In this report, CAT activity declined with raising Cd stresses that in conformity with the results of the preceding study on *Pistacia vera* (Rohani et al., 2019). Reduction in

CAT activity along with enhancing Cd concentration in some plant species can be referred to as the toxic influences of Cd or may be due to the decrease in protein content in consequence of the metal toxicity and oxidative stress or existent proteases in peroxisomes (Vajpaee et al., 2000). APX scavenge H_2O_2 via activating the glutathione ascorbate cycle. In various plant organelles like cytosol and plastid, APX can be found and it may be caused to exact ROS variations for signaling (Vajpaee et al., 2000; Rohani et al., 2019).

Under stress conditions, the amount of thiobarbituric acid reacting substances (TBARs) such as MDA is evaluated as lipid peroxidation index (Gapińska et al., 2008). MDA has an important function in abiotic stress. Lipid peroxidation in plant cells begins with several ROSs. Under environmental stresses, free radical levels increased in plants, and also membrane injury was investigated by measuring MDA content (Akbari et al., 2013). Moreover, estimation of MDA content is usually applied to determine the membrane lipid peroxidation size as a symptom of oxidative stress (Delavari et al., 2010).

The results of this experiment indicated that under Cd stress, BC usage increased *O. ciliatum* growth. In agreement with the findings of this research, the growth of several plants increased by BC usage under metal stress conditions (Rehman and Ahmad, 2016; Younis et al., 2016) or Cd stress (Abbas et al., 2017). Moreover, the preceding report indicated that BC application increased the wheat growth compared with the treatments with no BC application (Abbas et al., 2017) which is in conformity with the results of this experiment. This finding showed that BC application moderate negative influences of Cd stress in *O. ciliatum*.

Under Cd stress the plant growth decreased; while in this condition BC usage increased *O. ciliatum* growth due to the reduction in Cd concentrations by the so-called dilution effect of BC (Yousaf et al., 2016; Yu et al., 2019; Khan et al., 2020a, 2020b). In conformity with our results, in preceding studies chlorophyll content in the plants increased by BC application under metal stress (Younis et al., 2016; Abbas et al., 2017). Increasing chlorophyll content by BC usage might be due to the inversion of Cd-induced toxicity in the plant species (Rizwan et al., 2016b). Based on the previous reports, due to the sorptive properties of BC by decreasing plant exposure to stress agents or by enhancing the stress reactions of the plant species, BC acts to alleviate the harmful influences of plant stress (Beesley et al., 2011; Khan et al., 2020b; Kwon et al., 2020). Therefore, the capacity of BC for increasing water availability could be reduced the harmful effects of Cd which is reported in this study. BC adsorb significant amounts of ions like Cd in the soil. On the other hand, BC characteristics like porosity and specific surface area that influenced by pyrolysis conditions and types of raw materials that affected BC quality to enhance soil-plant water connection (Case et al., 2012; Khan et al., 2020b). In a few studies, physiological reactions of plants to BC usage have been evaluated. Describing the mechanism of BC is an important topic that is connected to growth enhancement and different influences. Until now the mechanism of BC unknown well, but based on the preceding research, the origin of biological activity is reported as BC reforestations (Bączek-Kwinta, 2017). Furthermore, the water-holding capacity of the soil is enhanced by the BC application. Therefore, the soil moisture availability enhancement is observed which can dilute ions in soil solution (Akhtar et al., 2015). So, by using BC EL reduced.

There is little knowledge concerning the BC effect on protein absorption. In the present study, the BC usage decreased soluble protein content maybe because BC decreases organic N cycling of the soil by reducing the production of protein and its use by plants (Prommer et al., 2014). Furthermore, this reduction probably due to the absorption of proteins and some enzyme inhibitors to the surfaces of BC (Rizwan et al., 2016c). Some defense systems present in the plants under stress conditions for protecting them from the negative influences of oxidative stress. One important factor to evaluate the cellular injury range produced in the stress conditions is the stability of the cell membrane. The results of previous studies showed that MDA accumulation increased under Cd stress and BC application caused a reduction in MDA contents in spinach (Younis et al., 2016) and wheat (Abbas et al., 2017) which are in agreement with the results of this experiment. Based on the previous research on wheat, application of BC increased antioxidant enzyme activities in comparison without using BC, and so oxidative stress decreased (Abbas et al., 2017) which is in agreement with the results of this report may be as a result of the lower Cd concentrations in the plant species by using BC.

Metal accumulation in plants connected to plant characteristics such as special organs of the species in addition to the heavy metals and their toxicity interaction (Rohani et al., 2019). In Cd-contaminated soils, the key to safe food production is the reduction in Cd content in edible parts of the plants (Rizwan et al., 2016c). In this experiment, at 2% w/w BC usage, the Cd concentration in the *O. ciliatum* leaves was below the maximum allowed Cd levels (0.2 mg/Kg dry weight; FAO, 2012). In agreement with our findings, Cd concentration decreased by using BC in some plants like spinach (Younis et al., 2016; Khan et al., 2020a), rice (Rizwan et al., 2018), and wheat (Abbas et al., 2017; Muhammad et al., 2020). The type of Cd in the soil was modified by BC which might reduce its uptake by plant species (Rizwan et al., 2016c; Yin et al., 2016; Lu et al., 2017; Jing et al., 2019; Qiu et al., 2020). Recently, Lomaglio et al. (2018) indicated that the wood BC application can decrease Cd portability, availability, and therefore its uptake in various parts of the bean. For Cd uptake by plants, bioavailable forms are more prominent than total Cd content in the soil (Rizwan et al., 2016c; Yin et al., 2016; He et al., 2019). Exchangeable Cd forms in the soil are reduced by BC usage while it is enhanced by Fe and Mn oxide bound structure (Yin et al., 2016). Reduction in Cd concentration after BC usage may be in consequence of the decrease in pore water Cd concentration or increasing in Cd levels bound to the soil organic matter (Lu et al., 2017).

Totally, evaluation of the previous experiments showed that BC amendment can decrease the detrimental influence of heavy metals (Jing et al., 2019; Qiu et al., 2020; Khan et al., 2020a, 2020 b; Muhammad et al., 2020) may be due to the different mechanisms comprise the enhancing of harmful ions absorption by BC usage, increasing the water-holding capacity of the soil and moisture availability of soil which can dilute ions in the soil solution that lead to a decrease in ion toxicity to the crops (Akhtar et al., 2015).

Heavy metals accumulation in the plants grown on the contaminated soils can increase human health risks. According to the results of the previous experiment, BC reduced the accumulation of heavy metals in different plant species, so decreased health risks and improves plant quality (Khan et al., 2020a, 2020b; Muhammad et al., 2020). A useful variable for estimation of the risks related to the special polluted plant consumption with heavy metals is THQ. For human health consumption, THQ lower than 1demonstrate no risk (Adel et al., 2016). Some important factors include element concentration; amount and type of them in the ingested food which is connected with the element absorption from consumed food (Zhu et al., 2013). Based on the WHO suggestion, restriction Cd consumption in the plants is 6 μm/kg. Moreover, as a result of the United Nations Food and Agriculture Organization (FAO) and WHO recommendation, short-term tolerable weekly intake Cd is 7 μm/kg per body weight (Raju et al., 2006; Arceusz et al., 2011). In this study, low Cd levels in daily consumption showed no risk to human health. Furthermore, the results of this study showed that using BC in all treatments decreased the THQ due to the absorption properties of BC and decreasing Cd levels of the plants. This means that BC usage in contaminated soil helps to reduce pollutions and decreases human risk assessment. Consequently, based on these properties of BC, the main mechanism for reducing stress is the sorption of Cd that causes to decrease in the plant response to metal stress. Thus BC can be applied as a soil amendment for producing safety products and minimizing health risks of heavy metals associated with the dietary intake plants cultivated in metal contaminated soils.

5. Conclusion

The findings of the present experiment showed that growth, biochemical and physiological characteristics of *O. ciliatum* as well as Cd content of the leaves are influenced by different Cd concentrations. As the authors indicated in the results, *O. ciliatum* exhibited different reactions to Cd stress and BC usage in the soil, especially at 2% w/w, alleviate negative effects of Cd stress on all of the studied traits of *O. ciliatum* significantly. In this study, the authors understand that BC can absorb ions such as Cd and decrease the negative effects of Cd stress. The findings of the present research showed that BC usage particularly 2% can protect *O. ciliatum* from Cd stress by mitigating the harmful effects of Cd as well as alleviating oxidative stress and nutrient balancing. BC decreased the bioavailability of Cd in the soil and its concentration in the leaves of *O. ciliatum*. BC usage in this study reduced leaf Cd concentrations of *O. ciliatum* under Cd stress probably in consequence of BC-induced enhance the growth of the plant and reduced bioavailability of Cd in the soil. Therefore, under Cd stress, BC increases the growth of *O. ciliatum* as well as reduction the danger for human health in Cd-contaminated soils and reduces THQ under Cd stress conditions. On the other side, due to the BC characteristics as an environmental friendly soil amendment, the authors suggested further experiment is required to estimate the capacity of different levels of BC to enhance the growth of plant under Cd stress, BC effects on Cd concentrations in various medicinal and edible plants that simultaneously contaminated with different Cd concentrations and discover new methods for decreasing the negative influences of Cd stress on different plant species.

CRediT authorship contribution statement

Leila Mehdizadeh designed the research, Writing - review & editing. **Sara Farsaraei** designed the research, and review and editing. **Mohammad Moghaddam** designed the research, Writing - review & editing. All authors read and approved the final manuscript.

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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