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Beneficial effects of biochar application on lettuce (*Lactuca sativa* L.) growth, root morphological traits and physiological properties

Dilfuza Jabborova◆*,, Dilbar Kadirova***, Abdujalil Narimanov* and Stephan Wirth****

**Institute of Genetics and Plant Experimental Biology, 111208 Kibray, Uzbekistan*
***Leibniz Centre for Agricultural Landscape Research (ZALF), 15374 Müncheberg, Germany*
****Termez State University, 190111, Termez, Uzbekistan*

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Abstract

Biochar application is widely used to improve plant growth by increasing soil fertility. This study aimed to assess the effect of biochar on lettuce growth, root morphological traits and physiological properties under greenhouse conditions. The study was performed in a greenhouse, using biochar additions of 1%, 2% and 3% in a pot experiment. The plant parameters assessed under greenhouse conditions were the germination rate, leaf length, leaf number, leaf width as well as fresh root weight, fresh shoot weight, dry root weight, dry shoot weight, and root morphological traits. Furthermore, chlorophyll content and relative water content were evaluated. Both biochar 2% and biochar 3% treatment ssignificantly improved lettuce growth (leaf length, leaf number, leaf width) compared with the control, while no significant differences were observed in the biochar 1% treatment. However, biochar 3% treatment significantly enhanced the root morphological traits such as total root length, the root surface area, the projected area and the root volume compared to the control. The total chlorophyll content and carotenoid content improved with increasing levels of biochar 3% treatment, which significantly increased by 43% and 51% compared with the control. The highest acid phosphomonoestrase activities were seen with the application of biochar 3%, which was 19% and 31% higher than that of the biochar 1% and control treatments, respectively. Moreover, a significant increase in soil enzyme activities (fluorescein diacetate hydrolase, acid and alkaline phosphomonoestrase) was observed with the biochar 2% and biochar 3% treatments compared to the control. This study indicated that biochar could promote lettuce growth and enhance soil enzyme activities.

1. Introduction

Lettuce (*Lactuca sativa* L.) is an annual plant of the aster family, Asteraceae and one of the most important leafy vegetable mainly used for fresh consumption or commonly served as salad applications (Labeda *et al*., 2007; Chiesa *et al*., 2009). This vegetable has several health benefits attributed to the presence of high levels of phyto-nutrients, high contents of vitamins K and A, beta-carotene as well as fiber content, phenolic compounds, and minerals such as calcium, phosphorus, potassium, manganese and iron (Mulabagal *et al*., 2010; Mulabagal *et al*., 2010; Jaiswal, 2020; Xylia *et al*., 2021). For successful cultivation, lettuce requires ample water and nutrient supply, especially in warmer conditions.

Biochar is a coal-like material obtained by pyrolysis of biomass which is used as soil improvement in agriculture (Kookana *et al*., 2011; Adrados *et al*., 2015). Many reports have shown that biochar application improved microbial activity, soil physical and chemical properties (Asai *et al*., 2009; Lehmann *et al*., 2011; Hale *et al*., 2013; Martinsen *et al*., 2015). Recently, Laird *et al.* (2010) reported that the biochar application enhanced the specific surface area, water holding capacity and cation exchange capacity. Scisowsk *et al*., (2015) showed that the application of biochar had a positive effect on soil quality and productivity. Jabborova *et al.* (2020) demonstrated that biochar improved contents of soil nutrients such as total nitrogen, potassium and phosphorus. Furthermore, several researchers reported that biochar improved soil enzyme activities such as dehydrogenase, alkaline and acid phosphatase, acid and alkaline phosphomonoesterase, proteases, lipase-esterase, and esterase (Anderson *et al*., 2011; Jabborova *et al*., 2021a; Jabborova *et al*., 2021b; Jabborova *et al*., 2021c). Soil catalase activity, phosphatase activity and urease activity were higher in the treatment with the highest application rate of biochar treatment (Oladele *et al*., 2019).

Biochar application can play an important role in different crops growth, yield, plant nutrients and plant physiological properties (Jabborova *et al*., 2020; Hashem *et al*.. 2019; Jabborova *et al*., 2021d). Vaccari *et al.* (2015) demonstrated that biochar treatments could enhance vegetative plant growth such as plant height, stem diameter, root dry weight and root length in tomatoes. Vaughn *et al.* (2020) found that 5% organic biochar and 10% organic biochar had increased seed germination and fresh weights per plant of tomato plants.Tomoto leaf samples concentrations of nitrogen (N), potassium (K), magnesium (Mg) and phosphorus (P) were also significantly higher in biochar treatments (Vaccari *et al*., 2015). The application of biochar significantly enhanced cabbage seedling production as reported by Chrysargyris *et al.* (2019). Numerous studies have revealed that

Corresponding author: Dr. Dilfuza Jabborova
Associate Professor, Institute of Genetics and Plant Experimental Biology, Kibray-111208, Uzbekistan
E-mail: dilfuzajabborova@yahoo.com
Tel.: +91-8595092177

biochar application improved plant photosynthesis, chlorophyll content, transpiration rate and relative water content (Speratti *et al*., 2018; Jabborova *et al*., 2021b; Jabborova *et al*., 2021d; Yoo *et al*., 2021). Thus, the relative water content, yield, and quality of tomatoes significantly increased by biochar application (Akhtar *et al*., 2014). The biochar amendment significantly increased the photosynthetic rate in okra (*Abelmoschus esculentus* L.) as reported by Sarma *et al*. (2017). Individual applications of biochar significant increase in leaf numbers and leaf areas in faba bean (*Vicia faba* L.), reported by Nahhas *et al*. (2021). Biochar treatments increased the chlorophyll a, chlorophyll b, and total photosynthetic pigments in chickpea (Hashem *et al*., 2019). Accordingly, the contents of chlorophyll a and b as well as total chlorophyll content of lettuce significantly increased at a biochar application of 7.5% (Chrysargyris *et al*. 2020).

However, detailed information on the effects of biochar on the morphological and physiological characteristics of lettuce leaves and roots is still lacking. The objectives of this study were thus to reveal the impacts of biochar application on lettuce growth and plant physiological properties, as well as on root morphological traits and soil enzyme activities in more detail.

2. Materials and Methods

2.1 Surface sterilization and germination of seeds

Lettuce seeds were sorted to eliminate broken, tiny, or infected seeds and sterilized with 10% sodium hypochlorite solution for three minutes and washed three times with sterile, distilled water. Lettuce seeds were germinated in 85 mm x15 mm tight-fitting plastic Petri dishes with 5 ml of water. The dishes were placed in incubator at temperature 28-30ºC.

2.2 Experimental design

The impact of biochar levels on the growth of lettuce was investigated in a greenhouse at ZALF, Müncheberg, Germany.The treatments included control (soil without biochar) and soil with three levels of biochar (1%, 2% and 3%). The lettuce was grown for 35 days at 24 °C day and 16ºC at night. The experiments were carried out in a randomized block design with five replications. Seed were sown into plastic pots, including 400 g of soil. All pots were watered every three days. The germination rate, leaf length, leaf number and width, fresh root and fresh shoot weight, dry root and dry shoot weight were measured after 35 days.

2.3 Measurement of root morphological traits

The lettuce roots were washed thoroughly and the entire root system was spread out and analyzed using a scanning system (Expression 4990, Epson, CA).The digital images of the root system were finally analyzed using the Win RHIZO software (Régent Instruments, Québec, Canada). Thus, the total root length, the root surface area, the root volume, the projected area and the root diameter were analyzed.

2.4 Measurement of physiological parameters

Total chlorophyll content and carotenoid contents were analyzed by the modified method of Hiscox and Israelstam (1979). Fifty mg fine pieces of 2 to 3 mm size fresh leaf sample were cut and added to test tubes containing 5 ml DMSO. Then the test tubes were incubated at 37°C for 4 h in the dark. The incubation was continued until a completely colorless tissue was obtained. The absorbance of the extract was taken at 470 nm, 645 nm and 663 nm using a spectrophotometer against DMSO blank.

The relative water content was determined by the method of Barrs and Weatherly (1962). One hundred mg fully expanded fresh leaf sample (FW) was placed immediately after sampling in Petri plates filled with double distilled water for 4 h at room temperature. The samples were then taken out, blotted, dried and the turgid weight (TW) was recorded. Then, the samples were kept in an oven at 70ºC overnight, and finally dry weight was recorded (DR). The relative water content was calculated as: RWC (%) = [(FW–DW) / (TW–DW)] X 100.

2.5 Determniation of soil enzyme activities

Soil enzymes such as the acid and alkaline phosphatase activities were evaluated by the method of Tabatabai and Bremner (1969).The fluorescein diacetat hydrolytic activity was analyzed by the method of Green *et al*. (2006). In brief, a total of 0.5 mg soil was added to 25 ml of sodium phosphate (0.06 M; pH 7.6). 0.25 ml of 4.9 µM fluorescein diacetate substrate solution was added to all assay vials. Each vial was mixed and incubated in a water bath at 37°C for 2 h. The soil suspension was centrifuged at 8000 rpm for 5 min. The clear supernatant was measured at 490 nm against a blank reagent solution in a spectrophotometer (Spectra Max Plus 384).

The phenoloxidase (POX) activity was determined following the method of Floch *et al.* (2007). A modified universal buffer (MUB) stock solution was prepared according to Tabatabai (1994) by dissolving 12.1 g of tris (hydroxymethyl) aminomethane (THAM), 11.6 g of maleic acid, 14.0 g of citric acid, and 6.3 g of boric acid (H_3BO_3) in 488 ml of a 1 M sodium hydroxide (NaOH) and diluting the solution to 1 litre with bidistilled water. Then, 200 ml of the MUB stock solution was titrated to the desired pH by using 0.1 M hydrochloric acid (HCl) or 0.1 MNaOH, and the volume was adjusted to 1 liter with bidistilled water. An ABTS (2,22 - azinobis-(-3ethylbenzothiazoline-6-sulfononic acid) diammonium salt stock solution was made by dissolving 0.548 g ABTS in 10 ml of bidistilled water for a final concentration of 0.1 M ABTS. POX activity was measured by spectrophotometer (Spectra Max Plus 384) using ABTS as the substrate. The reaction mixture contained: 1.0 g of soil, 10 ml of MUB solution pH 4.0 and 200 µl of a 0.1 M ABTS solution. The ABTS concentration in the incubation mixture was 2 mM. After incubation at 30°C for 5 min, the mixture was centrifuged at 12 000 rpm at 4°C for 2 min, and the supernatant was measured at 420 nm.

2.6 Statistical analyses

Experimental data were analyzed with the Stat View Software using one-way ANOVA. The significance of the treatment effect was determined by the magnitude of the F value (p<0.05, <0.001).

3. Results

3.1 Germination of seed

The biochar application levels of 1%, 2% and 3% biochar improved seed germination. Both biochar amendment of 1% and 2% enhanced the seed germination after three and four days compared to the control. Biochar amendment of 3% increased the seed germination by 100% compared to the control on days three and four (Figure 1).

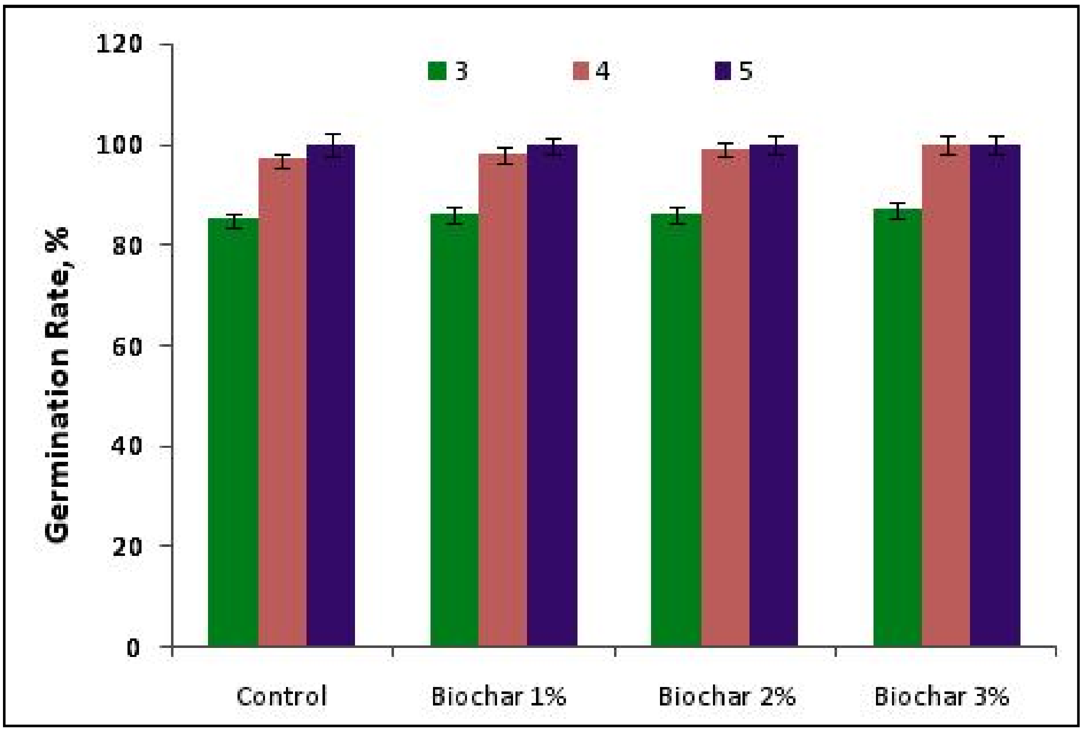

Figure 1: Effects of biochar amendments (1%, 2%, 3%) on the germination rate of lettuce after 3-5 days.

Figure 2: Lettuce growth under greenhouse conditions.

Table 1: Effect of biochar amendment (1%, 2%, 3%) on leaf length, leaf number and leaf width of lettuce

Treatments	Leaf length (cm)	Leaf number	Leaf width (cm)
Control	3.60 ± 0.55	5.20 ± 0.45	2.84 ± 0.17
Biochar 1%	4.04 ± 0.11	5.80 ± 0.45	3.22 ± 0.19
Biochar 2%	4.50 ± 0.21*	6.20 ± 0.45*	3.82 ± 0.15*
Biochar 3%	5.02 ± 0.11*	6.40 ± 0.55*	4.12 ± 0.18**

Data are means of five replicates (n = 5), asterisks indicate significant differences from the control at p<0.05,< 0.01.

3.2 Plant growth parameters of lettuce

Biochar addition increased leaf length, leaf number and leaf width after 35 days, Table 1 and Figure 2. Both biochar 2% and biochar 3% treatments showed a positive effect on leaf length compared to the control and 1% treatment. Leaf length exhibited that biochar treatments of 2% and 3% enhanced leaf length by 25 and 39% over the control. Compared to the control, biochar treatments of 2% and 3% improved the leaf number by 19% and 23%, respectively. The leaf width enhanced sharply with increasing levels of biochar additions of 2 and 3%, which significantly improved by 34% and 45% compared to the control (Table 1).

There were significant effects of biochar amendment of 1% on the root fresh weight and the root dry weight (Table 2). Compared to the control, the fresh root weight enhanced significantly by 35% and 43% for biochar treatments of 2% and 3%, respectively. Accordingly, both biochar treatments significantly enhanced the root dry weight by 37% and 50%. The biochar amendments of 2 and 3% also significantly increased the shoot fresh weight by 33% and 45% over the control and significantly increased the shoot dry weight by 24% and 38%.

3.3 Measurement of root system parameters

The root system of lettuce are presented in Figure 3. There was no significant biochar effect of the 1% treatment on the total root length, the root surface area, the projected area and the root volume. However, biochar 1% treatment enhanced the root diameter by 22%. Overall, biochar 2% and 3% treatments improved root morphological traits of lettuce at maximum compared to the control treatment. The root surface area improved significantly by 22% and 31% with increasing levels of biochar 2 and 3% treatments, compared with the control. For instance, biochar 2% and 3% treatments significantly increased the projected area by 28% and 38% compared with the control, while the root diameter enhanced with increasing levels of biochar treatments by 55% and 67% and the root volume by 37% and 55%. The total root length in the biochar 3% treatment was 39% higher than control, respectively (Table 3).

3.4 Determination of plant physiological traits

The influence of biochar levels on the total chlorophyll content of lettuce under greenhouse conditions is presented in Figure 2. The biochar 1% treatment slightly increased the chlorophyll content, however, the highest total chlorophyll content was with the biochar 3% treatment, which was 17% and 43% higher than for the biochar 1% and control treatments. In the treatment with 2% biochar application, the total chlorophyll content raises substantially by 33% compared to the control.

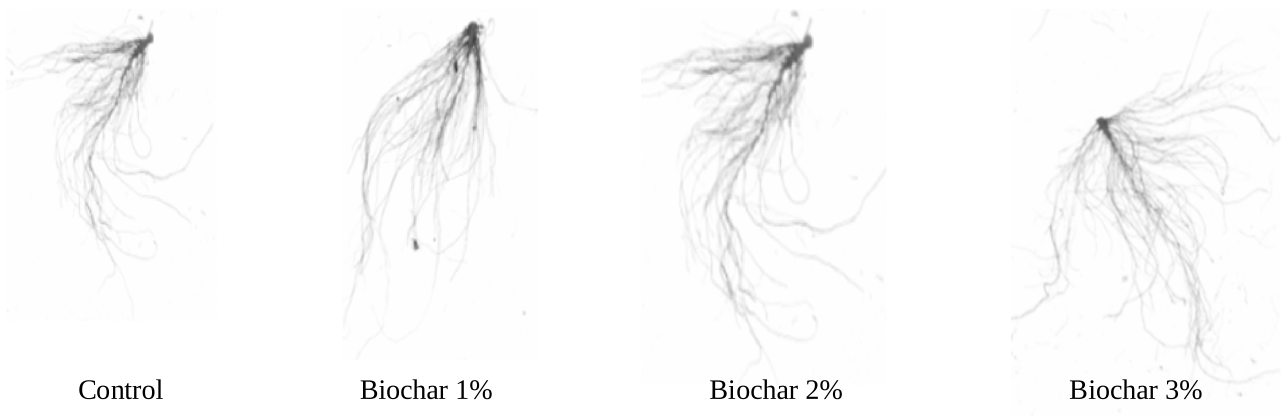

Figure 3: Effects of biochar amendments (1%, 2%, 3%) on the root system of lettuce.

Table 2: Effect of biochar amendment (1%, 2%, 3%) on fresh root and shoot weight and on dry root and shoot weight of lettuce

Treatments	Root fresh weight (g)	Shoot fresh weight (g)	Root dry weight (g)	shoot dry weight (g)
Control	2.81 ± 0.01	8.10 ± 0.07	0.16 ± 0.01	0.71 ± 0.02
Biochar 1%	3.34 ± 0.01*	9.48 ± 0.04	0.19 ± 0.01*	0.78 ± 0.01
Biochar 2%	3.80 ± 0.02*	10.80 ± 0.07*	0.22 ± 0.01*	0.88 ± 0.02
Biochar 3%	4.02 ± 0.03**	11.72 ± 0.04**	0.24 ± 0.01**	0.98 ± 0.01*

Data are means of five replicates (n = 5), asterisks indicate significant differences from the control at $p< 0.05, < 0.01$.

Table 3: Effect of biochar amendment (1%, 2%, 3%) on root morphological traits of lettuce

Treatments	Total root length (cm)	Root surface area (cm^2)	Projected area (cm^2)	Root diameter (mm)	Root volume (cm^3)
Control	122.3 ± 2.52	9.50 ± 0.10	10.90 ± 0.10	0.18 ± 0.01	0.38 ± 0.01
Biochar 1%	133.0 ± 3.53	10.8 ± 0.06	12.5 ± 0.20	0.22 ± 0.01*	0.44 ± 0.01
Biochar 2%	143.0 ± 6.56*	11.57 ± 0.25*	13.97 ± 0.15*	0.28 ± 0.01*	0.52 ± 0.02*
Biochar 3%	170.6 ± 1.97*	12.43 ± 0.31*	15.10 ± 0.26	0.30 ± 0.01**	0.58 ± 0.02**

Data are means of five replicates (n = 5), asterisks indicate significant differences from the control at $p< 0.05, < 0.01$.

Table 4: Effects of biochar amendment (1%, 2%, 3%) on soil enzyme activities

Treatments	Acid phosphomono-esterase (μg g-1 h-1)	Alkaline phospho monoesterase (μg g-1 h-1)	Fluorescein diacetateactivity (μg g-1 h-1)	Phenol oxidase activity (U.g-1 DW)
Control	900.2 ± 26.6	576.6 ± 15.0	35.2 ± 0.65	20.1 ± 0.35
Biochar 1%	993.4 ± 28.2	645.5 ± 13.2	40.5 ± 2.65	27.6 ± 1.10*
Biochar 2%	1085.0 ± 30.1*	656.4 ± 12.7	42.6 ± 1.52*	31.3 ± 1.65**
Biochar 3%	1178.8 ± 37.3*	689.1 ± 14.4*	44.8 ± 3.69*	34.5 ± 1.80**

Data are means of five replicates (n = 5), asterisks indicate significant differences from the control at $p< 0.05,< 0.01$.

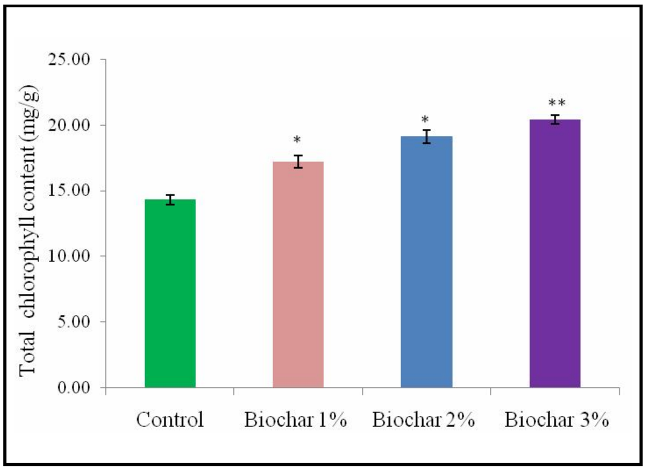

Figure 2: Effects of biochar amendments (1%, 2%, 3%) on the total chlorophyll content of lettuce.

Data are means of five replicates (n = 5), asterisks indicate significant differences from the control at $p<0.05$.

The results in Figure 3 show that the carotenoid content of lettuce was substantially enhanced in treatments with 1% and 3% biochar. The biochar 1% and 2% treatments significantly increased the carotenoid content by 24% and 38%, respectively. The highest carotenoid content was seen with biochar 3% treatment which was 17% and 51% higher than the biochar 1% and control treatments, respectively.

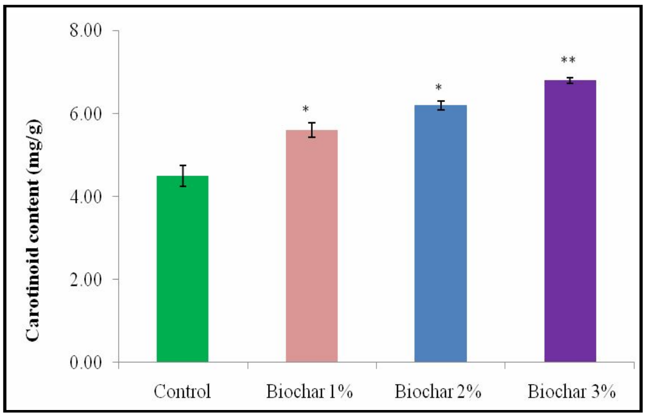

Figure 3: Effects of biochar amendments on the carotenoid content of lettuce.

Data are means of five replicates (n = 5), asterisks indicate significant differences from the control at $p<0.05$, <0.01.

Furthermore, biochar treatments increased the relative water content of lettuce. The treatments with 1% and 2% biochar slightly increased the relative water content in leaf tissues compared to the control, whereas the maximum relative water content was detected in the treatment with 3% biochar compared with control.

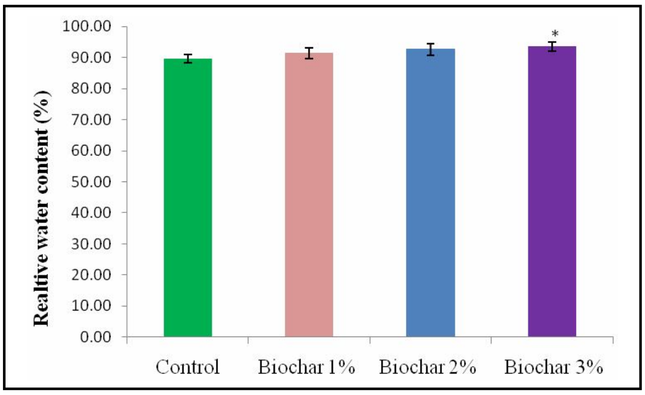

Figure 4: Effects of biochar amendments (1, 2%, 3%) on the relative water content of lettuce leaf tissues.

Data are means of five replicates (n = 5), asterisks indicate significant differences from the control at $p<0.05$.

3.5 Estimation of soil enzyme activities

The biochar application significantly improved soil enzymes activities, however, except for the fluorescein diacetate, and the acid and alkaline phosphomonoesterase activities, no significant differences were observed in the biochar 1% treatment (Table 4). The highest alkaline phosphomonoesterase activities were seen with the application of biochar 3%, which was 19% higher compared to the control treatment, respectively. The fluorescein diacetate hydrolase and phenol oxidase activities were also significantly increased with biochar 3% treatment by 27% and 72%, compared to the control. The biochar 2% treatment significantly increased the acid phosphomonoesterase activity by 20% and the fluorescein diacetatehydrolase activity by 27% over the control. The phenol oxidase activity increased from 56% in biochar 2% treatment compared to the control.

4. Discussion

Previous reports indicated that the major effects of biochar application were based on enhancing seed germination, plant growth and finally yields of several crops such as chickpea (Hashem *et al.*, 2019), soybean (Ma *et al.*, 2019; Jabborova *et al.*, 2020), spinach (Jabborova *et al.*, 2021a) and okra (Jabborova *et al.*, 2021d). Several plant growth parameters and root morphological traits of lettuce were significantly increased by biochar 2% and 3% treatments compared to control. Correspondingly, Carter *et al.* (2021) reported that rice-husk biochar application increased plant biomass, root biomass, plant height and number of leaves of cabbage (*Brassica chinensis)* and lettuce (*Lactuca sativa*) compared to the control. This finding confirms earlier studies by Nabavinia *et al.* (2015) that biochar application increased the root growth, root diameter, and yield of radish (*Raphanus sativus*). Liang *et al.* (2019) recorded a higher *P.australis* biomass in biochar treatment.This finding is also consistent with Usman *et al.* (2016), who observed a significant increase in tomato (*Solanum lycopersicum*) vegetative growth and yield that increased 14.0-43.3% by biochar. According to Gale and Thomas (2019), biochar generally increased plant growth and the concentration of micronutrients in *Abutilon theophrasti* and *Trifolium repens*. Correspondingly, the application rate of 10-15% sludge biochar on the green roof exerted the most significant effects on plant biomass by 54.0-54.2% (Chen *et al*., 2018).

The addition of biochar enhanced root growth parameters and root morphological parameters such as the total root length, the root surface area, the projected area, the root diameter and the root volume compared to the control. Several researchers also found that biochar application enhanced root growth of different plants such as soybean (*Glycine max* L), ginger (*Zingiber officinale*) and also in case of basil (G³odowska *et al*., 2017; Ma *et al*., 2019; Jabborova *et al*., 2021b; Jabborova *et al*., 2021c).

In the present study, biochar 3% treatment had a positive effect on lettuce physiological properties such as total chlorophyll and carotenoid contents as well as the relative water content of leaf tissues. Accordingly, numerous researchers found that biochar application increased plant physiological traits in different plants such as chickpea (*Cicer arietinum*), ginger (*Zingiber officinale*), and also for okra (*Abelmoschus esculentus*) (Hashem*et al.,* 2019; Jabborova *et al.*, 2021b; Zhang *et al*., 2020; Jabborova *et al.*, 2021d). Similarly, biochar-amended a significant increase in the chlorophyll (41%) of soybean plants when grown on a soil compared to the control (Qian *et al*., 2019). Younis *et al.* (2016) showed that biochar increases chlorophyll a by 29%, chlorophyll b by 52%, total chlorophyll by 33%, and carotenoids by 5% in spinach (*Spinacia oleracea*). Similarly, results were reported by Zeeshan *et al.* (2020), who observed that biochar application significantly enhanced plant height, fresh and dry weight, chlorophyll *a*, *b*, total chlorophyll and carotene content.

Furthermore, our results indicated that biochar 3% treatments significantly increased the activities of soil enzymes such as phenol oxidase, fluorescein diacetate hydrolase acid and alkaline phosphomonoesterase. These finding confirm earlier studies such as by Chen *et al.* (2013) who observed significant increase in alkaline phosphatase activity in soil amended with wheat straw biochar. Accordingly, enhanced soil enzyme activities was reported by Elzobair *et al.* (2016) after the addition of biochar. Earlier studies by Jabborova *et al.* (2020) and Jabborova *et al.* (2021b) who both observed the fluorescein diacetate hydrolase acid and alkaline phosphomonoe strase activities of the soil in biochar application. Biochar application significantly increased the phosphatase activity by 46% biochar application with the highest rate 12 t ha (Oladele. 2019), biochar was 34%. Similarly, Saeed *et al.* (2021) recorded the soil enzymatic activities had shown 38.5% increase in fluorescein diacetate hydrolysis and 55.6% increase in dehydrogenase activity in soil incubated with biochar in compared to control.

5. Conclusion

Both biochar treatments of 2% and biochar 3% significantly increased lettuce growth such as leaf length, leaf number and leaf width compared to the control. Therefore, biochar application at 3% was rated to be sufficient for achieving the best root morphological traits such as total root length, root surface area, projected area and root volume. Moreover, biochar application at 3% increased the total chlorophyll content and carotenoid content. Soil enzyme activities (fluorescein diacetate hydrolase, acid and alkaline phosphomonoesterase) were further increased by increasing rates of biochar application. Thus, results clearly indicated improved growth conditions of lettuce and soil enzyme activities after biochar amendment.

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Conflicts of interest

The authors declare no conflicts of interest relevant to this article.

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