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Growth Response of Ginger (*Zingiber officinale*), Its Physiological Properties and Soil Enzyme Activities after Biochar Application under Greenhouse Conditions

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Abstract: This study aimed to investigate the effects of biochar (1%, 2%, and 3%) on seed germination, plant growth, root morphological characteristics, and physiological properties of ginger (*Zingiber officinale*) and soil enzymatic activities. Pot experiments under greenhouse conditions at 24 °C (day) and 16 °C (night) showed after six weeks that biochar additions of 2% and 3% significantly increased seed germination, plant height, leaf length, leaf number, as well as shoot and root dry weights compared to the control. Total root length significantly increased by 30%, 47%, and 74%, with increasing biochar contents (1%, 2%, and 3%) compared to the control. Root surface area, projected area, root diameter, and root volume reached a maximum at the 3% biochar treatment. The treatment with 2% biochar significantly increased fluorescein diacetate hydrolase and phenoloxidase activities by 33% and 59% compared to the control; so did the addition of 3% biochar, which significantly increased fluorescein diacetate hydrolases, phenoloxidase, and acid and alkaline phosphomonoesterase activity in soil compared to the control. Treatment with 3% biochar increased relative water content by 8%, chlorophyll content by 35%, and carotenoid content by 43% compared to the control. These results suggest that biochar can improve the performance of the rhizome of ginger and increase the activity of soil enzymes, thereby improving soil nutrient supply.

Keywords: Ginger (*Zingiber officinale*); biochar; plant growth; root morphological traits; chlorophyll content; soil enzymes

1. Introduction

Ginger (*Zingiber officinale* Rosc.) is an important herb and spice plant belonging to the Zingiberaceae family. The rhizome of ginger is significant for health and is considered effective against several ailments or disease-related manifestations such as headaches, nausea, vomiting, and motion sickness [1–3]. In addition, ginger is attributed with antitumorigenic and immunomodulatory effects. Furthermore, it serves as an antimicrobial, antiviral agent, is considered a potent analgesic and stimulant, and controls various diseases such as high cholesterol and blood pressure [4,5]. Ginger is used to treat a wide range of ailments such as stomach pain, diarrhea, nausea, asthma, and respiratory diseases [6]. The production of ginger as a nutrient-exhaustive crop requires an adequate supply of nutrients [7].

Mineral fertilizers and also biochars of various origins and qualities have been used to increase the yield of crops. Biochar contributes to decrease global warming, reduces atmospheric CO_2 concentrations, increases soil organic carbon (SOC), and improves soil nutrients [8,9]. Soil organic carbon increases after biochar application [10]. Accordingly, Scisłowska et al. [11] reported that biochar treatments improved the quality and productivity of soils. The biochar treatments significantly increased water holding capacity, cation exchange capacity, and specific surface area [12]. Biochar has also been reported to positively affect plant growth, development, and yield of various plants. Several reports have explicitly found that biochar increased seed germination, plant growth, and yield of various plants [13–19]. Thus, biochar treatment increased root biomass and shoot biomass of *Plantago lanceolate* compared to the control [20]. Rice straw biochar significantly increased plant height, bolls per plant, average boll weight, and cotton yield compared to the control treatment [21].

The addition of biochar can improve the availability of plant nutrients as well as plant physiological properties. The concentrations of Ca and Mg in corn leaf samples were significantly higher at a high biochar application rate than in the control [13]. Several studies have shown that biochar application increases plant photosynthesis, chlorophyll content, and transpiration rate [15,16,22]. The addition of biochar significantly increased the photosynthetic rate of okra (*Abelmoschus esculentus* L.) [18]. Regarding soil biological processes, it has been reported that the availability of nutrients and the activity of soil enzymes and microbial biomass in the soil are influenced by biochar. Accordingly, the availability of soil nutrients such as K, Ca, Mg, Na, and total C was improved by biochar [23,24]. In addition, biochar application significantly promoted N content in studies by Saxena et al. [25]. Moreover, biochar treatment has been reported to increase enzyme activities such as proteases, phosphohydrolases, and esterases [16,26,27]. Biochar application is accompanied by increased microbial biomass carbon content [28]. However, the effects of biochar or biochar-based fertilizers on ginger yield and soil nutrient availability have not been widely examined [29]. The objective of this study was to investigate the effect of biochar application on the growth, root morphological characteristics, physiological properties, and soil enzymatic activities of ginger (*Zingiber officinale*) grown under greenhouse conditions.

2. Materials and Methods

The biochar used in the study was produced at 450 °C from black cherry wood (Terra Anima® Onlineshop, Meissen, Germany) and had a particle size of less than 4 mm. The biochar and soil properties are summarised in Table 1 [19]. Ginger seeds (*Zingiber officinale*) were purchased from a local market in Berlin, Germany. The effect of biochar content on ginger growth was investigated in pot experiments in a greenhouse at ZALF, Müncheberg, Germany. All experiments were conducted in a randomized block design with three replicates. The experimental treatments included the control (soil without biochar) and soil with three levels of biochar (1%, 2%, and 3%). Plants were grown under greenhouse conditions at 24 °C during the day and 16 °C at night for 6 weeks. The seeds were cultivated in plastic pots (12 cm diameter, 18 cm depth) with 1.5 kg soil. Each pot was watered every 3 days. At harvest after 6 weeks, the germination rate, plant height, leaf length, leaf number, and leaf width, root and shoot fresh weight, and root and shoot dry weight were measured. The roots were carefully washed with water. The entire root system was then spread out and analysed with a scanning system (Expression 4990, Epson, Los Alamitos, CA) using a blue board as background. The digital images of the root system were analysed using Win RHIZO software (Régent Instruments, Quebec, QC, Canada). The total root length, root surface area, root volume, projected area, and root diameter were evaluated.

Table 1. Soil and biochar characteristics.

	Total (g kg^{-1})			Available (mg kg^{-1})				C/N	pH (H_2O)
	C	N	S	Ca	K	Mg	P		
Biochar	415.0	3.75	0.58	8893	1151	471	326	110.6	8.41
Soil	9.18	0.99	0.23	2103	1080	954	419	9.26	6.26

The relative water content (RWC) was measured according to the method of Barrs and Weatherley [30]. One hundred mg of fully expanded fresh leaf samples (FW) were placed in Petri dishes filled with double-distilled water for 4 h at room temperature immediately after sampling. The samples were then removed, blotted dry, and the threshold weight (TW) was recorded. The samples were then stored overnight in an oven at 70 °C, and the dry weight (DR) was recorded. The relative water content was calculated as:

$$RWC\ (\%) = [(FW - DW)/(TW - DW)] \times 100$$

Photosynthetic pigments were determined according to a modified method of Hiscox and Israelstam [31]. Freshly cut leaf samples of fifty mg pieces each, 2 to 3 mm in size, were placed in test tubes containing 5 mL DMSO. The test tubes were then incubated at 37 °C for 4 h in the dark. Incubation was continued until the tissue was completely colourless. The absorbance of the extract was measured at 470 nm, 645 nm, and 663 nm with a spectrophotometer against DMSO blank. The contents of chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll and carotenoids were determined using the following equation:

$$\text{Total Chl (mg/g)} = [20.2\ (A645) + 8.02\ (A663)] \times V/W$$

$$\text{Carotenoids (mg/g)} = [(1000 \times A470) - (3.27 \times \text{Chl a} + 104 \times \text{Chl b})] \times V/W$$

where A = optical density; V = volume of DMSO (in ml); W = sample weight.

The acid and alkaline phosphatase activities were determined according to the method of Tabatabai and Bremner [32]. The hydrolytic activity of FDA was determined according to the method of Green et al. [33]. For this, a total of 0.5 mg of soil was mixed with 25 mL of sodium phosphate (0.06 M; pH 7.6). Then, 0.25 mL of a 4.9 mM FDA substrate solution was added to all test vials. All vials were mixed and incubated for 2 h in a water bath at 37 °C. The bottom suspension was then centrifuged at 8000 rpm for 5 min. The clear supernatant was measured at 490 nm against a reagent blank solution in a spectrophotometer (SpectraMax Plus 384).

Phenol oxidase (PO) activity was determined according to the method of Floch et al. [34]. Here, a modified universal buffer (MUB) stock solution was prepared according to Tabatabai [35], by dissolving 12.1 g tris(hydroxymethyl)aminomethane (THAM), 11.6 g maleic acid, 14.0 g citric acid, and 6.3 g boric acid in 488 mL 1 M sodium hydroxide (NaOH) and diluting the solution to 1 L with double-distilled water. Then 200 mL of the stock MUB solution was titrated to the desired pH with 0.1 M hydrochloric acid (HCl) or 0.1 M NaOH, and the volume was made up to 1 L with double-distilled water. An ABTS (2,2′- azinobis-(-3ethylbenzothiazoline-6-sulfononic acid) diammonium salt stock solution was prepared by dissolving 0.548 g ABTS in 10 mL double-distilled water for a final concentration of 0.1 M ABTS. PO activity was measured spectrophotometrically (SpectraMax Plus 384) with ABTS as substrate. The reaction mixture contained: 1.0 g soil, 10 mL MUB solution pH 4.0, and 200 µL of a 0.1 M ABTS solution. The final 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 for 2 min and the rate of oxidation of ABTS to ABTS+- released in the supernatant was measured at 420 nm.

The experimentally determined data were analysed with StatView software using 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. Plant Growth and Root Morphological Traits of Ginger

Biochar application generally increased seed germination compared to the control. Seed germination increased 8 days faster with the application of 2% and 3% biochar compared to the control (Figure 1). Both levels of biochar amendment (2% and 3%) significantly increased seed germination up to 95–98% on day 8 compared to the control. All three levels of biochar amendments increased seed germination to 100% on day 11 compared to the control.

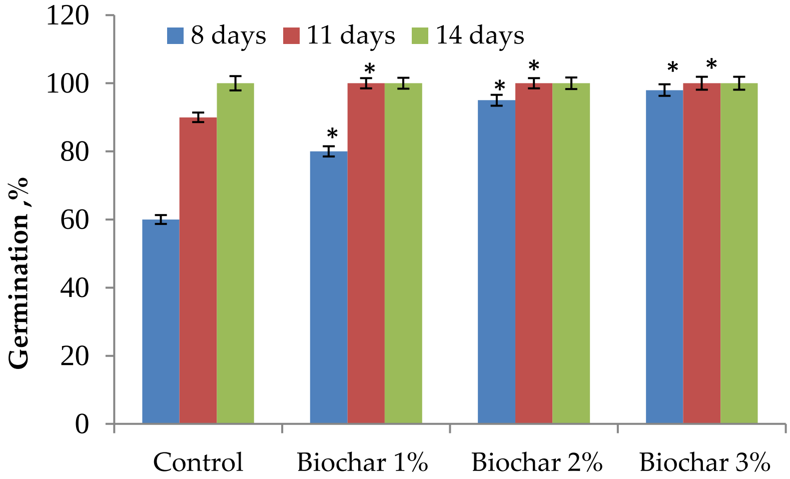

Figure 1. The impact of biochar concentrations on germination of ginger. Data are means of three replicates (n = 3), asterisks indicate significant differences from the control at $p < 0.05$. The 2% biochar treatment increased plant height by 53% compared to the control, and the 3% biochar treatment showed a 78% increase compared to the control treatment. The 2% and 3% biochar treatments also showed that leaf length increased by 36% and 57%, respectively, compared to the control. The leaf number increased significantly with increasing addition of biochar up to 85% compared to the control (Table 2). The leaf width also improved significantly with the addition of 2% and 3% biochar by 54% and 73% as compared to the control.

Table 2. The impact of biochar concentrations on plant height, leaf length, leaf number, and leaf width of ginger.

Treatments	Plant Height (cm)	Leaf Length (cm)	Leaf Number	Leaf Width (cm)
Control	18.0 ± 0.27	5.8 ± 0.01	3.3 ± 0.01	1.1 ± 0.01
Biochar 1%	22.3 ± 0.22	6.9 ± 0.03	4.0 ± 0.02	1.5 ± 0.01 *
Biochar 2%	27.5 ± 0.18 *	7.9 ± 0.01 *	4.8 ± 0.1 *	1.7 ± 0.01 *
Biochar 3%	32.1 ± 0.72 **	9.1 ± 0.03 *	6.1 ± 0.02 **	1.9 ± 0.01 **

Data are means of three replicates (n = 3), asterisks indicate significant differences from the control at $p < 0.05$, <0.01.

The shoot and root dry weights showed that the biochar treatments improved both the shoot and the root dry weight compared to the control (Figure 2). The 1% biochar treatment significantly increased shoot dry weight by 51% compared to the control. Treatment with 2% and 3% biochar significantly increased shoot dry weight by 67% and 79% compared to control. Root dry weight also increased sharply with increasing biochar, with a significant 67% increase at 2% biochar compared to the control (Figure 2). Root dry weight reached a maximum at 3% biochar treatment, with root dry weight significantly improved by 79% compared to the control.

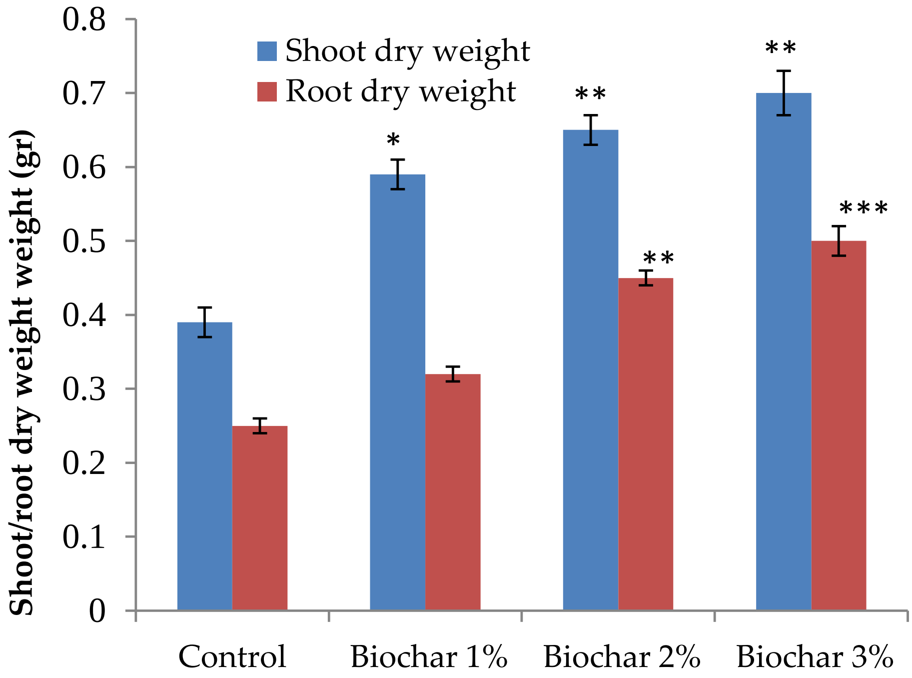

Figure 2. The impact of biochar concentrations on shoot and root dry weight of ginger. Data are means of three replicates (n = 3), asterisks indicate significant differences from the control at $p < 0.05$, <0.01, <0.001.

The data regarding the morphological characteristics of the roots showed that the total root length, root surface area, projected area, root diameter, and root volume increased with increasing addition (1%, 2%, and 3%) of biochar compared to the control (Table 3). Thus, total root length significantly increased by 30%, 47%, and 74% compared to the control, and root surface area significantly increased by up to 90% compared to the control (Table 3). The projected area reached a maximum at the 3% biochar treatment with an increase of 95% compared to the control. Root diameter improved greatly with the increasing amount of biochar by up to 97% compared to the control, as did root volume, which increased by up to 88% compared to the control (Table 3).

Table 3. The impact of biochar concentrations on root morphological traits of ginger.

Treatments	Total Root Length (cm)	Root Surface Area (cm^2)	Projected Area (cm^2)	Root Diameter (mm)	Root Volume (cm^3)
Control	114.1 ± 2.05	30.9 ± 1.12	23.0 ± 0.11	1.06 ± 0.01	1.17 ± 0.01
Biochar 1%	149.5 ± 2.83 *	46.7 ± 1.09 *	29.7 ± 0.13	1.20 ± 0.01	1.43 ± 0.01
Biochar 2%	167.8 ± 10.11 *	50.3 ± 2.00 **	38.8 ± 1.00 **	1.62 ± 0.02 *	1.68 ± 0.02 *
Biochar 3%	198.4 ± 12.00 **	58.6 ± 2.08 ***	44.8 ± 1.07 ***	2.09 ± 0.02 ***	1.99 ± 0.01 ***

Data are means of three replicates (n = 3), asterisks indicate significant differences from the control at $p < 0.05$, <0.01, <0.001.

3.2. Physiological Properties of Ginger

The data also showed that, depending on the concentration, the biochar treatments also improved the relative water content compared to the control. With increasing addition of biochar, the water content increased significantly by up to 8% compared to the control after 3% biochar amendment (Figure 3).

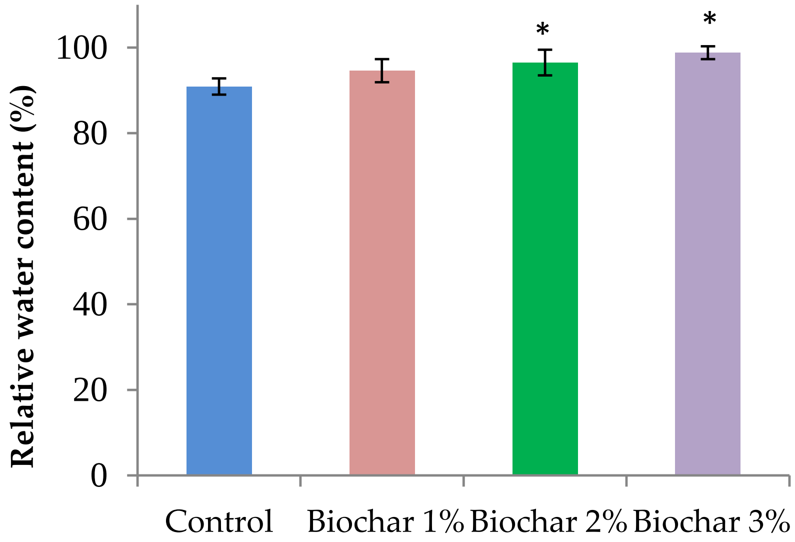

Figure 3. The impact of biochar concentrations on the relative water content of ginger. Data are means of three replicates ($n = 3$), asterisks indicate significant differences from the control at $p < 0.05$.

The data showed that the 2% biochar treatment significantly increased chlorophyll content by 23% compared to the control. The 3% biochar treatment showed a further increasing positive effect by 35% compared to the control (Figure 4).

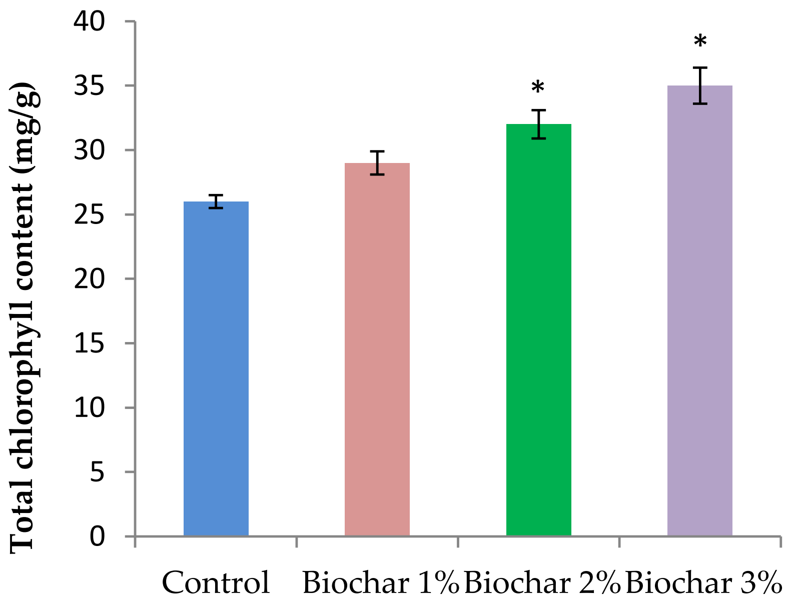

Figure 4. The impact of biochar concentrations on chlorophyll content of ginger. Data are means of three replicates ($n = 3$), asterisks indicate significant differences from the control at $p < 0.05$.

In addition, the results on carotenoid contents showed that the biochar additions of 2% and 3% significantly increased the carotenoid content by 30% and 43%, respectively, compared to the control (Figure 5).

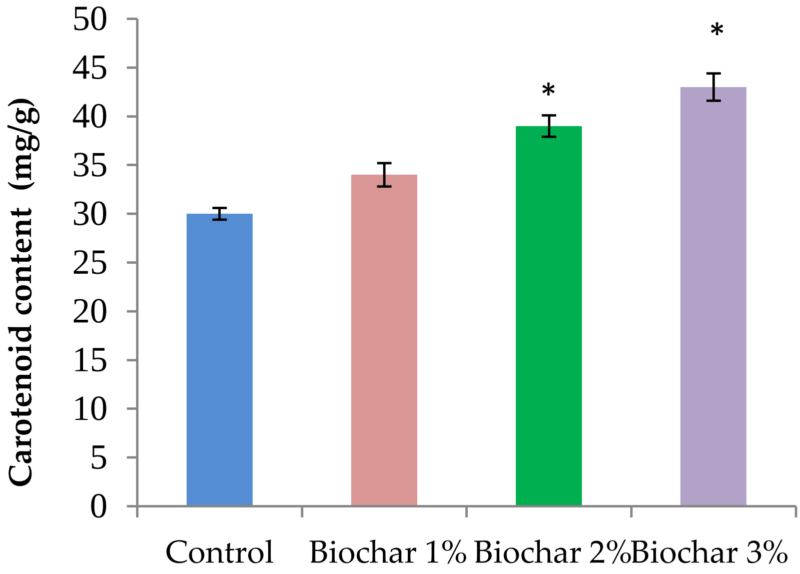

Figure 5. The impact of biochar concentrations on carotenoid content of ginger. Data are means of three replicates ($n = 3$), asterisks indicate significant differences from the control at $p < 0.05$.

3.3. Soil Enzyme Activities

The addition of biochar increased the activities of fluorescein diacetate hydrolases, phenol oxidases, and acidic and alkaline phosphomonoestrases in soil. The data in Table 4 show that 1% biochar treatment significantly increased phenol oxidase activity by 38% compared to the control, and the 2% addition significantly increased fluorescein diacetate hydrolase activity and phenol oxidase activity by 33% and 59% compared to the control. Soil acidic and alkaline phosphomonoestrase activity reached a maximum at the 3% biochar treatment compared to the control. The 3% addition of biochar increased the fluorescein diacetate hydrolase activity as well as the phenol oxidase activity by 55% and 77%, respectively, compared to the control.

Table 4. The impact of biochar concentrations on soil enzymes.

Treatments	Acid Phosphomono-Esterase ($\mu g\ g^{-1}\ h^{-1}$)	Alkaline Phosphomono-Esterase ($\mu g\ g^{-1}\ h^{-1}$)	FDA (Fluorescein Diacetate) Activity ($\mu g\ g^{-1}\ h^{-1}$)	Phenol Oxidase Activity ($U.g^{-1}$ DW)
Control	980.1 ± 36.6	656.7 ± 10.5	40.6 ± 1.19	22.1 ± 0.11
Biochar 1%	1099.8 ± 38.4	677.1 ± 12.6	45.7 ± 1.30	30.5 ± 0.17 *
Biochar 2%	1176.9 ± 43.0	698.2 ± 15.3	54.2 ± 1.12 *	35.2 ± 1.10 *
Biochar 3%	1238.4 ± 44.1 *	753.6 ± 20.1 *	62.8 ± 1.28 *	39.2 ± 1.02 **

Data are means of three replicates ($n = 3$), asterisks indicate significant differences from the control at $p < 0.05$, <0.01.

4. Discussion

In the present study, the control treatment reduced seed germination, plant height, leaf number, leaf length, leaf width of ginger. Accordingly, several studies have reported that ginger growth and yield significantly decreased without mineral fertilizer [36–40], proving that ginger is a plant with specific nutrient requirements.

In the present study, we used black cherry biochar at different concentrations to enhance seed germination and growth of ginger. Together, the concentrations of 2% and 3% biochar promoted maximum seed germination, which was significantly higher compared to the control. Accordingly, Kanwal et al. [41] reported that the addition of 1% and 2% biochar increased the germination of wheat seeds. Moreover, this result is in agreement with the report of Bu et al. [42], who observed a significant increase in germination rate, the dry matter of shoots and roots of *Robinia pseudoacacia* L. seeds by rice husk biochar and wood chips biochar. Similar results confirming improved germination of castor seeds by the addition of 1% and 5% castor stem biochar were reported by Hilioti et al. [43]. The positive effect of biochar addition on seed germination, root length, shoot length, root biomass, shoot biomass, and yield has also been reported by Saxena et al. [25]. Jabborova et al. [44] reported that biochar application significantly increased shoot length, leaf lengths, leaf number, leaf width, root dry weight, shoot dry weight, and total root length. This result is in agreement with the report of Carter et al. [14], who observed that application of biochar from rice husks increased final biomass and root biomass of lettuce (*Lactuca sativa*) and cabbage (*Brassica chinensis*) compared to treatment without biochar. In terms of plant height, leaf length, leaf number, width shoot dry weight, and root dry weight, it was shown that the 2% and 3% biochar treatments significantly increased these plant growth parameters compared to the control. Thus, several other researchers reported that biochar increased plant growth, root dry matter, shoot dry matter and yield of numerous other crops [13,15,17,20,45].

Root morphological parameters such as total root length, root surface area, projected area, root diameter, and root volume were improved by biochar. In the present study, root morphological characteristics such as total root length, root surface area, projected area, root diameter, and root volume were observed to be improved by biochar additions of 2% and 3% compared to the control. A number of other studies have also reported that biochar application improved plant root growth and development [17,27,46]. A significant increase in root length, root surface area, and root volume at an application rate of 1% rice husk biochar and woodchip biochar has also been reported by Bu et al. [42]. The results of this study showed that the 3% biochar treatment significantly increased root diameter and projected area compared to all treatments. In the present study, ginger physiological properties such as chlorophyll content, carotenoid content, and relative water content were indicated to be improved by biochar additions of 2% and 3% compared to the control without biochar. Similar results were reported by other authors [12,15,16], where the biochar application increases plant photosynthesis, chlorophyll content, and transpiration rate. The application of biochar significantly increased the content of chlorophyll a, chlorophyll b, total chlorophyll, carotenoid contents, and relative water of spinach (*Spinacia oleracea* L.) [44]. Chrysargyris et al. [47] reported that trees biochar at 7.5% and 15% significantly increased total chlorophylls content of lettuce. Our study further showed that biochar treatment positively affected plant physiological parameters, such as chlorophyll content and carotenoid content, which reached a maximum of 3% biochar treatment compared to the control. Similarly, Sarma et al. [18] reported a pronounced positive effect of biochar addition on the photosynthetic rate of okra. Other researchers found that biochar application increased chlorophyll content, transpiration rate, total flavonoids, sugars, and glucose in various plants in addition to photosynthesis [15,22,48]. Corresponding results confirming a significant increase of 27.1% in photosynthetic rate and 16.1% in chlorophyll concentration by biochar addition were reported by He et al. [49]. The activity of a number of soil enzymes was promoted by biochar application. In the present study, fluorescein diacetate hydrolase, phenoloxidase, and soil acidic and alkaline phosphomonoesterase activities reached a maximum with 3% biochar treatment compared to the control and the other biochar treatments. Thus, other authors also reported that biochar applications increased soil enzymes such as proteases, phosphohydrolase, lipases, and esterases [16,26,27]. This result also confirms studies by Bailey et al. [50] and Ma et al. [19], both of which observed increased soil enzyme activity upon biochar application, suggesting increased soil microbiological activity. Similar results were confirmed after biochar application by Wang et al. [51]. A significant increase in urease activity by 40%, invertase activity by 9%, and phosphatase activity by 46% with biochar application has been reported by Oladele [52]. Thus, increased activity of soil enzymes due to the addition of biochar also contributed to better availability of nutrients in the soil [53].

5. Conclusions

This study found that biochar application improved seed germination, plant height, leaf length, leaf number, leaf width, as well as shoot and root weight of ginger. The most effective treatment of 3% biochar application also significantly increased root dry weight, shoot dry weight, and root morphological characteristics. The 3% biochar treatment also had a pronounced, positive impact on soil enzyme activity, relative water content, chlorophyll content and carotenoid content as compared to all other treatments. Thus, the present study indicates that biochar application is a promising option to improve and stabilize soil fertility, yield, and quality criteria in ginger cultivation.

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