Commagene Journal of Biology

Sezen & Küçük, (2024) *Comm. J. Biol.* 8(2), 80-86.
DOI: 10.31594/commagene.1527214

e-ISSN 2602-456X

Research Article/Araştırma Makalesi

Effects of Biochar and *Cladophora glomerata* on Wheat (*Triticum aestivum* L.) Growth and Rhizosphere Enzyme Activities

Göksal SEZEN*, Çiğdem KÜÇÜK

Harran University Faculty of Arts and Sciences Department of Biology Şanlıurfa, TÜRKİYE
ORCID ID: Göksal SEZEN: https://orcid.org/0000-0001-9054-851X; Çiğdem KÜÇÜK: https://orcid.org/0000-0001-5688-5440

Received: 02.08.2024 **Accepted:** 22.10.2024 **Published online:** 07.11.2024 **Issue published:** 31.12.2024

Abstract: The positive effects of biochar on both soil quality and plant growth and also on plant growth of macroalgae have been reported in studies. Studies on biochar and macroalgae interaction are quite limited. This study was carried out according to randomized plot design in greenhouse conditions to determine the effects of biochar and *Cladophora glomerata* applications and interaction on the growth of wheat (*Triticum aestivum* L.) and some enzyme activities in the rhizosphere. Biochar and *C. glomerata* interaction increased wheat root (90%) and shoot dry weight (84.2%), root length (43.1%) and plant height (84.2%) compared to control. Biochar application increased alkaline phosphatase activity by 66.3%, while *C. glomerata* increased β-glucosidase activity by 49%. The interaction of both applications increased catalase activity by 62.1% compared to control. These findings confirm the potential of biochar and *C. glomerata* to improve wheat production by inducing growth.

Keywords: Plant growth, *Cladophora glomerata*, biochar, soil enzymes.

Biyokömür ve *Cladophora glomerata'nın* Buğday (*Triticum aestivum* L.) Gelişimi ve Rizosfer Enzim Aktivitelerine Etkileri

Öz: Biyokömürün hem toprak kalitesi hem de bitki gelişimine ayrıca makroalglerin bitki gelişimi üzerine olumlu etkileri yapılan çalışmalarda rapor edilmiştir. Biyokömür ve makroalg interaksiyonu ile ilgili çalışmalar ise oldukça sınırlıdır. Bu çalışma, biyokömür ve *Cladophora glomerata* uygulamaları ve interaksiyonunun; buğdayın (*Triticum aestivum* L.) gelişimi ve rizosferdeki bazı enzim aktiviteleri üzerindeki etkisini belirlemek için serada koşullarında tesadüf parselleri deneme desenine göre yürütülmüştür. Biyokömür ve *C. glomerata* interaksiyonu kontrolle karşılaştırıldığında buğdayın kök (%90) ve sürgün kuru ağırlığını (%84.2), kök uzunluğunu (%43.1) ve bitki boyunu (%84.2) kontrole göre artırmıştır. Biyokömür uygulaması alkalin fosfataz aktiviteyi %66.3 oranında arttırırken, *C. glomerata* β-glukosidaz altiviteyi %49 oranında artırmıştır. Her iki uygulamanın interaksiyonu katalaz aktiviteyi kontrolle karşılaştırıldığında %62.1 oranında artırmıştır. Bu bulgular, biyokömür ve *C. glomerata*'nın buğday gelişimini tetikleyerek üretimini iyileştirme potansiyelini doğrulamaktadır.

Anahtar kelimeler: Bitki gelişimi, *Cladophora glomerata*, biyokömür, toprak enzimleri.

1. Introduction

The use of organic amendments affects soil organic matter that impacts many important physicochemical and biological processes in the soil (Danish et al., 2011). Soil organic matter is an important component of the soil system and is continuously depleted, especially in semi-arid and arid regions (Diacono and Montemurro, 2010). The stability of organic matter has been attributed to the ability of carbon-containing nutrients added to soils to provide stable organic carbon (Song et al., 2020). The application of organic material can lead to a decrease in soil oxygen, increased evaporation of NH_3, immobilization of soil mineral nutrients, and the production of phytotoxic compounds (Diacono and Montemurro, 2010). In this respect, biochar provides an organic impetus to the sustainability of agriculture and the environment (Chen et al., 2020). In some cases, the incorporation of solid charred biomass (biochar) into the soil has been described to increase soil fertility and carbon stocks over long periods of time (Hu et al., 2024). Algal extracts can be used as natural plant growth stimulants in sustainable and organic plant production (Mahmoud et al., 2019). *Cladophora glomerata* is a green macroalga found in both marine and freshwater (Pikosz et al., 2016). It is considered harmful in eutrophic water reservoirs as it can increase biomass up to three times a day (Messyasz et al., 2015). Although considered an environmental problem, *C. glomerata* has been used in various applications due to the abundance of carotenoids (Michalak and Messyasz, 2021). Studies have identified the positive effect of macroalgal extracts on plant growth, including *Sargassum vulgare* (brown macroalgae) (Mahmoud et al., 2019), *Codium taylorii* (green macroalgae), and *Pterocladia capillacea* (red macroalgae) (Kassim et al., 2016). Macroalgae in the form of extracts or dry biomass were able to influence plant growth, which was particularly evidenced by the positive effect of *Ulva fasciata* (green) and *Sargassum laserifolium* (brown) on radish growth and yield (Ahmed et al., 2021)

Enzymes are important factors in the microbial activities of soil microbial communities, which are important for the biogeochemical cycle in the soil ecosystem (Aponte et al., 2020). Therefore, soil enzymes have an important role in soil organic matter transformation, nutrient release, and fertility maintenance (Goncalves-Lopes et al., 2021). By measuring soil enzyme activities, information on soil chemical properties, fertility

Corresponding author: sezen@harran.edu.tr

levels, microbiological properties, and soil pollution status can be obtained (Jiang et al., 2021). Studies to determine the effects of biochar and *C. glomerata* on microbial parameters in soils are very limited. Therefore, in this study, the effects of biochar and *Cladophora glomerata* separately and in interaction on wheat growth and enzymatic activities of rhizosphere soil under greenhouse conditions were investigated.

2. Material and Method

2.1. Materials used in the experiment

Soil samples taken from the campus area where no previous application was made were sieved through a 2 mm sieve and filled into 3 kg pots. The soil used in the experiment has a pH of 7.2, organic matter (%) 1.22; lime (%) 2.38; EC (mmhos/cm) 1.54; N 0.14%, and has a clayey texture. *C. glomerata* used in the experiment was collected from Euphrates River in Karkamış District of Gaziantep in July 2023. Invertebrates (mussel and snail shells) in the algae were cleaned from stones and sand and dried in the shade. They were then pulverized with a Tefal Ultra High Speed Blender (shredder) at 46 000 rpm and stored in a deep freezer at -20 ^{o}C. Biorfe brand biochar was used as biochar. Biochar applied to the soil was added to the soil according to the instructions for use (250 g of biochar to 25 liters of soil). Wheat seeds (*Triticum aestivum* L.) were used in the experiment.

2.2. Set-up of the experiment

Wheat seeds were soaked in 10% v/v sodium hypochlorite (NaOCl) for two minutes, then washed with 70% ethanol, then with sterile distilled water, and excess water was removed on filter paper. The soil was mixed with the amount of biochar determined according to the instructions for use. *C. glomerata* was prepared at the rate of 2% in the root zone of the plant after germination and 100 ml/kg of soil was given to each pot twice with 15 days intervals. The experiment was set up as follows: a) plants grown in soil without biochar and *C. glomerata* (control); b) plants grown in soil containing biochar; c) plants grown in soil containing *C. glomerata* and without biochar; d) Plants grown in soil treated with *C. glomerata* and mixed with biochar. The experiment was conducted in three replications in a greenhouse with natural light. Plants were harvested 30 days after sowing. At the end of the harvest, plant height, green parts, root weights, and root length were determined according to Yıldız and Özgen (2004).

2.3. Determination of chlorophyll content of wheat leaves

Chlorophyll content of leaves was analyzed according to Arnon (1949). Leaf samples of each treatment were homogenized with acetone: water mixture and centrifuged at 5000x g. The absorbance of the filtrate was measured in a spectrophotometer at 645 nm and 663 nm wavelength in 3 replicates. The results were calculated in mg/l according to Arnon (1949).

2.4. Determination of some enzyme activities of rhizosphere

At the end of the harvest, soil samples adhering to the roots were taken, brought to the laboratory without waiting and analyzed. Alkaline phosphatase activity in soil was determined according to Tabatabai (1994). β-Glucosidase (EC3.3.1.21) activity was measured using p-nitrophenyl-β-D-glucopyranoside as substrate. In β-glucosidase activity, toluene was added to 0.5 g of soil sample, shaken for 15 minutes, then buffer solution and p-nitrophenyl-β-D-guloside solution were added and incubated at 37°C for 1 hour. Calcium chloride solution and tris solution (pH 12) were then added and the supernatant was measured spectrophotometrically at 410 nm after centrifugation (Yin et al., 2014). Catalase activity of rhizosphere soil was determined by Scheibler calcimeter. Phosphate buffer was added to the soil sample, H_2O_2 was placed in a glass tube, the jar was sealed with a stopper, and the oxygen output was recorded. The results were calculated as mg O_2 /5 g soil (Beck, 1971).

2.5. Statistical analysis

The results of the experiment were evaluated using the JMP11 statistical program for basic plant growth characteristics, rhizosphere catalase, alkaline phosphatase, and β-glucosidase activities.The results are given as the average of three measurements (n = 3) ± SE for each sample. Differences between treatments were determined using the Duncan Multiple Range Test.

3. Results and Discussion

3.1. Effects of applications on basic plant characteristics

The effects of *C. glomerata*, biochar treatments and the interaction of *C. glomerata* and biochar on root and shoot dry weight of wheat were examined and the results are given in Table 1. As a result of biochar and *C. glomerata* interaction, shoot wet and dry weight, root length, plant height, and root dry weight of wheat were significantly increased compared to control plants (Table 1). Co-application of *Cladophora glomerata* and biochar increased root dry weight by 78.9%, shoot dry weight by 62.8%, plant height by 26.7%, and shoot wet weight by 48.5% compared to the control.

Table 1. Effects of treatments on growth parameters of wheat

Treatments	Plant height (cm)	Root length (cm)	Shoot wet weight (g/plant)	Shoot dry weight (g/plant)	Root dry weight (g/plant)
Control	33 c*	6.07b	1.7b	0.13c	0.04c
C. glomerata (C)	44.33a	8.17a	3.28a	0.31a	0.09b
Biochar (B)	40.90b	7.77a	2.13ab	0.23b	0.07b
C+B	45a	8.67a	3.30a	0.35a	0.19a
LSD (p<0.05)	1.37	0.49	0.51	0.02	0.02

*Different letters in the same column are statistically significant (p<0.05).

Wheat plant height, root length, root and shoot weight were higher in *C. glomerata* and biochar treatments compared to control plants. Biochar has been reported to positively affect root-associated microbial diversity producing various metabolites that promote plant growth (Jiang et al., 2021). The increase in plant growth parameters of biochar and *C. glomerata* treatments compared to the control is thought to indicate that biochar improves the potential effect of plant beneficial microorganisms on plant growth through possible stimulation of the root system. In this study, it was explained that biochar addition to soil increased plant growth as it increased nutrient availability for microbial proliferation (Khan et al., 2020). Different formulations of macroalgae have also been used to increase the production of various plants (Baroud et al., 2021; Battacharyya et al., 2016; Hamouda et al., 2022). As a result of the studies, it has been determined that macroalgae extracts contain significant amounts of mineral nutrients, carbohydrates, amino acids, osmoprotectants, and antioxidants that contribute positively to plant growth (Ali et al., 2021; Battacharyya et al., 2015; Espinosa-Anton et al., 2023). Macroalgae have also been reported to contain various mucopolysaccharides, alditols, phenols, and organic substances that increase plant productivity and conserve soil moisture (Ma et al., 2022).

The treatments increased chlorophyll content. In a study, it was reported that *C. glomerata* treatment significantly increased the chlorophyll content of radish leaves compared to the control group (Dziergowska et al., 2021). In our study, the treatments significantly increased the chlorophyll content compared to the control (Fig. 1).

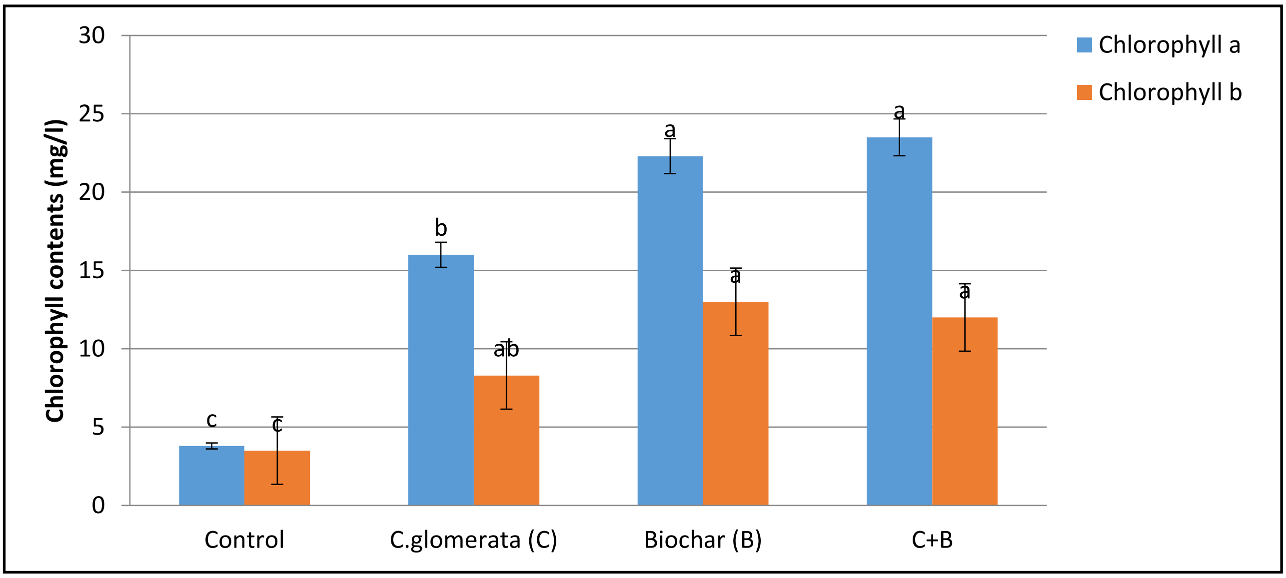

Figure 1. Effects of treatments on chlorophyll content in leaves (mg/l)

These results are similar to the increase in chlorophyll content of leaves examined in the treatment of *Codium taylorii* and *Pterocladia capillacea* extracts (Kassim et al., 2016), treatment of radish seeds with *Sargassum vulgare* extract (Mahmoud et al., 2019), *Ulva fasciata* and *Sargassum laserifolium*. The increase in chlorophyll content compared to the control may be due to the improvement in pigment biosynthesis, the content of phytohormones such as cytokinins, as well as the high concentrations of N and Mg (structural components of chlorophyll) in algal extracts. In maize plants, wheat straw biochar has been described to significantly increase plant height, chlorophyll content, water use efficiency, and grain weight due to an increase in soil P, K, N, and microbial biomass (Abbas et al., 2020). Biochar applications were found to improve pecan walnut tree height, chlorophyll content, photosynthetic rate, and N, P, Fe and K accumulation by increasing soil N content and enzyme activities (Hou et al., 2020).

3.2. Effects of applications on rhizosphere enzyme activities

Soil enzymes are important participants in the organic matter cycling and biochemical process of the soil system. Their activities are closely related to soil organic matter content, physical properties and microbial activities (Dounoras et al., 2024). Soil enzymes are one of the most active organic components in soil. They are important for the decomposition and nutrient cycling of soil material and determine soil nutrient availability and plant yield (Chen et al., 2023). It has been explained that catalase activity depends on the organic carbon content of the soil (Chen et al., 2020). In this study, it was determined that soil catalase activity increased with the addition of biochar and *C. glomerata* to the soil. As shown in Figure 2, the catalase activity of each treatment was higher than the control, indicating that the application of biochar and *C. glomerata* to the soil may be beneficial for increasing soil catalase activity. The addition of *C. glomerata* to the soil increased catalase activity by 54.2% compared to the control, while the addition of biochar increased catalase activity by 50%. In the interaction of *C. glomerata* and biochar, the activity increased by 62.1%. Our results showed that catalase activity increased in accordance with the reported results (Tu et al., 2020). The increase in catalase activities may be due to the increase in organic matter induced by the addition of biochar and *C. glomerata*, which provides sufficient substrate to promote microbial enzymatic reactions (Wang et al., 2019). This could be attributed to the coexistence of both beneficial and functional microorganisms, as nutrients and substrates in the soil were sufficient during the application period and the number of microorganisms in the soil gradually increased.

Phosphatases are produced by plants and microorganisms in soil (Chen et al., 2020). An increase in phosphatase activities was determined by adding biochar to the soil (Fig. 2). In terms of biochar addition, it is reported that the available carbon sources contained in biochar work together to accelerate microorganism growth in soil (Khan et al., 2019); thus, biochar promotes enzyme activities. Biochar plays an important role in maintaining microbial growth as well as providing energy (Paz-Ferreiro et al., 2013). The addition of biochar significantly increased the alkaline phosphatase activity of the soil as it brought active substances with it. The addition of biochar and *C. glomerata* to the soil increased the alkaline phosphatase activity compared to the control group (Fig. 3).

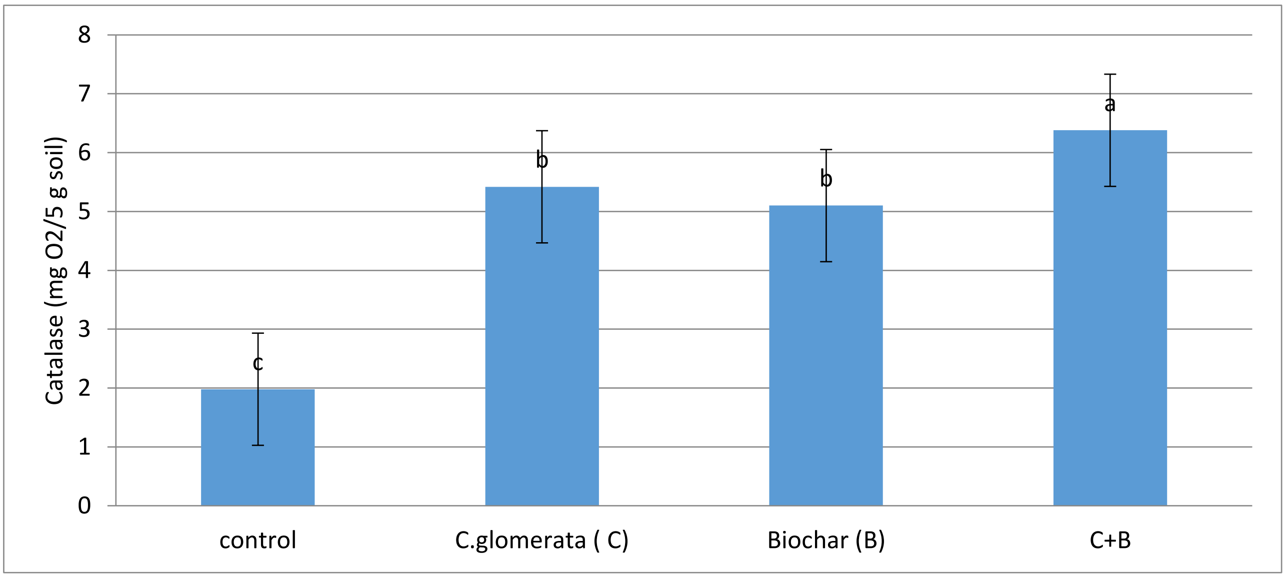

Figure 2. Effects of treatments on catalase enzyme activity of rhizosphere soil. The difference between means given with the same letter is not significant.

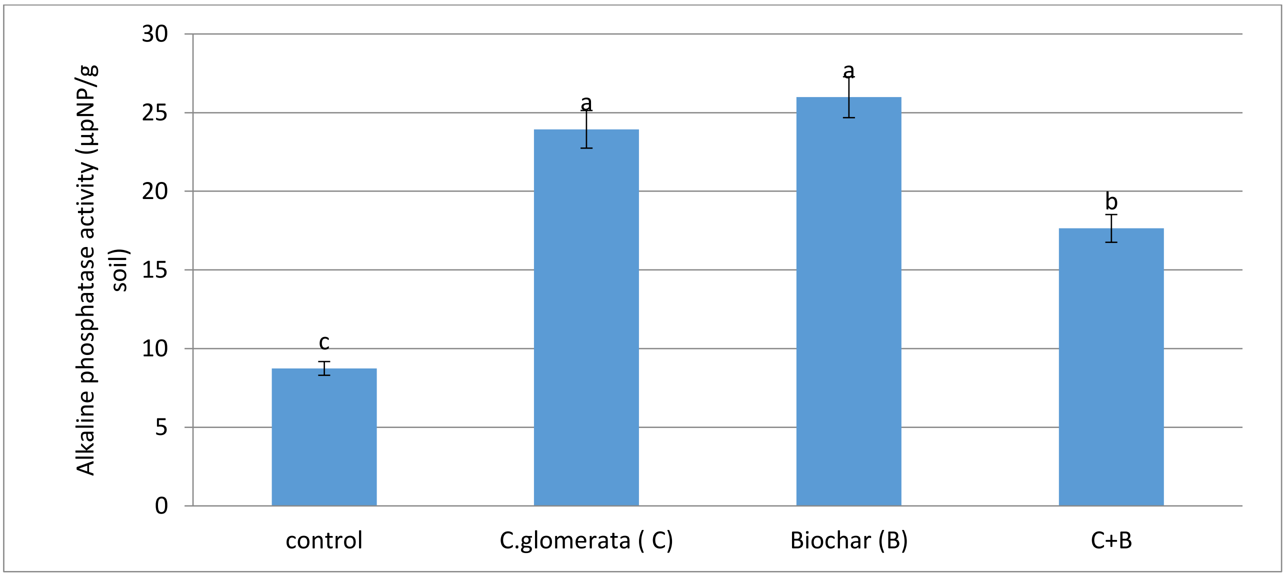

Figure 3. Effects of treatments on alkaline phosphatase enzyme activity of rhizosphere soil. The difference between means given with the same letter is not significant.

β-Glucosidase, urease, phosphatase, and sucrase are important for the conversion of soil nutrients. Among them, sucrase and glucosidase are associated with carbon conversion in soil and phosphatase can increase the availability of phosphorus. Agricultural management measures such as fertilization and irrigation can significantly affect soil enzyme activities (Chen et al., 2023). Our study shows that the β-glucosidase activity of *C. glomerata*-treated soil significantly increased compared to non-*C. glomerata*-treated soil (Fig. 4). The increase in enzyme activity promotes nutrient cycling and improves soil nutrient levels. It has been described that macroalgae can reduce nitrogen leaching and ammonia volatilization in soil and improve nitrogen use efficiency (Chen et al., 2023). As a plant-derived preparation, macroalgae are mainly composed of organic carbohydrates such as polysaccharides. Since *C. glomerata* is thought to be able to improve soil properties and microflora, it can increase soil enzyme activities and soil enzymes can actively participate in nutrient cycling, providing mineral nutrients for plants (Chen et al., 2023). The application of biochar in this study affected the activity rate of the β-glucosidase enzyme, which may be related to the increase in carbon availability promoted by biochar. β-glucosidase actively participates in the carbon cycle and is involved in the hydrolysis of organic products (Khan et al., 2019). Moreover, the presence of volatile compounds in biochar may have contributed to the enzyme activity (Liao et al., 2016). In this study, the findings of increased β-glucosidase activity due to the inclusion of biochar and *C. glomerata* in soil compared to the control are supported by previous studies (Ali et al., 2019, Song et al., 2020). The increase in β-glycosidase activity in soil may have contributed to the increase in the activity of other enzymes. β-glycosidase has been reported to release low molecular weight sugars, which are energy sources for microorganisms in soil, leading to increased microbial activity (Pathan et al., 2017).

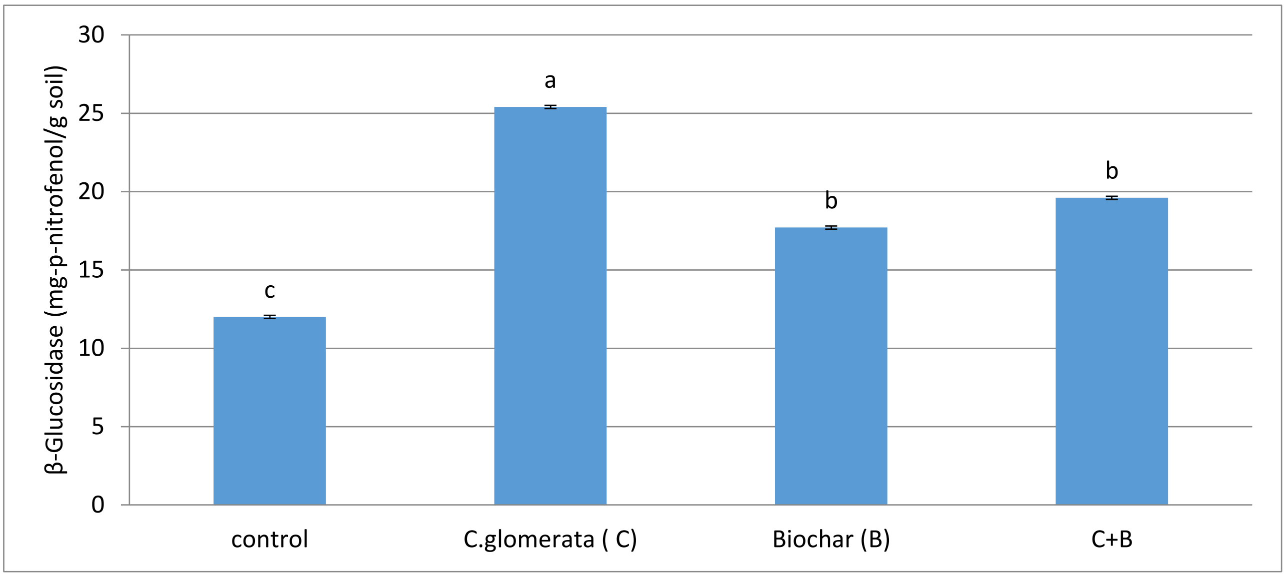

Figure 4. Effects of treatments on β-Glucosidase enzyme activity of rhizosphere soil. The difference between means given with the same letter is not significant.

Soil enzymes are indicators of soil quality as they are directly related to soil microbial activity and biogeochemical cycling of nutrients (Jiang et al., 2021). An increase in the activity of extracellular enzymes (β-glucosidase, β-xylosidase and β-d-cellobiosidase) involved in the sulfur and carbon cycle in soil has been recorded in biochar application (Jiang et al., 2021). Several studies (Goncalves-Lopes et al., 2021; Jiang et al., 2021; Tu et al., 2020) also revealed that biochar showed different effects on enzyme activities in different soil types. The application of macroalgae products in sufficient quantities has been described to indirectly affect the nutrient uptake capacity of plants by improving the physical, chemical, and biological properties of the soil or substrate (Ma et al., 2022). *Ulva ohnoi* was found to contain polyanionic compounds with strong chelating activity, such as phenolics and ulvan, which can form complexes with metal ions essential for plant nutrition (Illera-Vines et al., 2020). Similarly, amino acids (e.g., cysteine, glycine, histidine, and glutamic acid) contained in some macroalgae species have been found to bind to some trace elements to form very small and electrically neutral chelates, accelerating the uptake and transport of elements important in plant nutrition (Ma et al., 2022). Macroalgae applications have also been found to have positive effects on nutrient cycling and plant roots by promoting the growth of beneficial root-associated microorganisms (Popko et al., 2018). Metabolites produced by algae are used by microorganisms in soils as sources of nutrients and carbon, leading to an increase in the microbial population in the soil, and some microorganisms are capable of producing extracellular enzymes to hydrolyze organic P in soil that cannot be used by plants, which is thought to increase the efficient use of phosphorus by plants (Higo et al., 2020). However, further studies are needed to investigate the long-term effects of *C. glomerata* and biochar on soil microorganisms that benefit the soil in various ways and to reveal their ecological role in biochar-treated soils.

4. Conclusions

Biochar and *C.glomerata* treatments significantly increased the activities of rhizosphere enzymes. This may be due to the proliferation of beneficial microorganisms that enhance microorganism activities and enzymatic reactions. This study also showed that the addition of biochar and *C. glomerata* to the soil significantly improved phosphatase, catalase, and β-glucosidase activities compared to the control. The treatments were also found to have an effect on the main plant growth parameters and the results showed a synergistic effect of *C. glomerata* and biochar on plant growth and soil enzymes. These findings provide new insights into the potential of biochar and *C. glomerata* co-application on both plant growth and soil enzyme activities to improve wheat production. Our study demonstrated that biochar and soil-applied *C. glomerata* can be used as a sustainable amendment to enhance plant growth and improve soil quality, which will play a vital role in agriculture.

Acknowledgments: We would like to thank Ramazan AKTAN, a graduate student of Harran University, Department of Biology, and other undergraduate students who contributed to the experimental phase of this study.

Ethics committee approval: Ethics committee approval is not required for this study

Conflict of interest: The authors declare that there is no conflict of interest.

Author Contributions: Conception – G.S., Ç.K.; Design – G.S., Ç.K.; Supervision – G.S., Ç.K.; Fund – G.S., Ç.K.; Materials – G.S., Ç.K.; Data Collection and Processing – G.S., Ç.K.; Analysis Interpretation – G.S., Ç.K.; Literature Review – G.S., Ç.K.; Writing – G.S., Ç.K.; Critical Review G.S., Ç.K.

References

Abbas, A., Naveed, M., Azeem, M., Yaseen, M., Ullah, R., & Alamri, S. (2020). Efficiency of wheat straw biochar in combination with compost and biogas slurry for enhancing nutritional status and productivity of soil and plant. *Plants*, 9, 1516. https://doi.org/10.3390/plants9111516

Ahmed, D.A.E., Gheda, S.F., & Ismail, G.A. (2021). Efficacy of two seaweeds dry mass in bioremediation of heavy metal polluted soil and growth of radish (*Raphanus sativus* L.) plant. *Environmental Science and Pollution Research*, 28, 12831–12846. https://doi.org/10.1007/s11356-020-11289-8

Ali A., Guo D., Arockiam Jeyasundar P.G.S., Li Y., Xiao R., Du J., Li R., & Zhang Z. (2019). Application of wood biochar in polluted soils stabilized the toxic metals and enhanced wheat (*Triticum aestivum*) growth and soil enzymatic activity. *Ecotoxicology and Environmental Safety*, 184, 109635, https://doi.org/10.1016/j.ecoenv.2019.109635

Ali, O., Ramsubhag, A., & Jayaraman, J. (2021). Biostimulant properties of seaweed extracts in plants: Implications towards sustainable crop production. *Plants*, 10, 531. https://doi.org/10.3390/plants10030531

Aponte, H., Meli, P., Butler, B., Paolini, J., Matus, F., & Merino, C. (2020). Meta-Analysis of Heavy Metal Effects on Soil Enzyme Activities. *Science of the Total Environment*, 737, 139744. https://doi.org/10.1016/j.scitotenv.2020.139744

Arnon, D. (1949). Copper enzymes in isolated chloroplast polyphenol oxidase in Beta vulgaris. *Plant Physiology*, 24,1-15

Baroud, S., Tahrouch, S., El Mehrach, K., Sadki, I., Fahmi, F., & Hatimi, A. (2021). Effect of brown algae on germination, growth and biochemical composition of tomato leaves (*Solanum lycopersicum*). *Journal of the Saudi Society of Agricultural Sciences*, 20, 337–343. https://doi.org/10.1016/j.jssas.2021.03.005

Battacharyya, D., Babgohari, M.Z., Rathor, P., & Prithiviraj, B. (2015). Seaweed extracts as biostimulants in horticulture. *Scientia Horticulturae*, 196, 39–48. http://doi.org/10.1016/j.scienta.2015.09.012

Beck, T.H. (1971). Die Messung Katalasen Aktivitaet Böden. Z. Pflanzenernaehai. *Sodenk*, 130, 68-81.

Chen H., Yang X., Wang H., Sarkar B., Shaheen S. M., & Gielen G. (2020). Animal Carcass- and Wood-Derived Biochars Improved Nutrient Bioavailability, Enzyme Activity, and Plant Growth in Metal-Phthalic Acid Ester Co-contaminated Soils: A Trial for Reclamation and Improvement of Degraded Soils. *The Journal of Environmental Management*, 261, 110246. http://doi.org/10.1016/j.jenvman.2020.110246

Chen, D., Zhiming, L., Yang, J., Zhou, W., Wu, Q., Shen, H., & Ao, J. (2023). Seaweed extract enhances drought resistance in sugarcane via modulating root configuration and soil physicochemical properties. *Industrial Crops and Products*, 194, 116321. https://doi.org/10.1016/j.indcrop.2023.116321

Danish, S. A. Ameer, T.I. Qureshi, U. Younis, H. Manzoor, A. Shakeel, M. & Ehsanullah, M. (2014). Influence of biochar on growth and photosynthetic attributes of *Triticum aestivum* L. under half and full irrigation. *International Journal of Biosciences*, 5(7), 101-108 https://doi.org/10.12692/ijb/5.7.101-108

Daunoras, J., Kacergius, A., & Gudiukaite, R. (2024). Role of Soil Microbiota Enzymes in Soil Health and Activity Changes Depending on Climate Change and the Type of Soil Ecosystem. *Biology*, 13, 85. https://doi.org/10.3390/biology13020085

Diacono, M., & Montemurro, F. (2010). Long-term effects of organic amendments on soil fertility. A review. *Agronomy for Sustainable Development*, 30, 401-422. http://doi.org/10.1051/agro/2009040

Dziergowska, K., Wełna, M., Szymczycha-Madeja, A., Chęcmanowski, J., & Michalak, I. (2021). Valorization of *Cladophora glomerata* Biomass and Obtained Bioproducts into Biostimulants of Plant Growth and as Sorbents (Biosorbents) of Metal Ions. *Molecules*, 26, 6917. https://doi.org/10.3390/molecules26226917

Espinosa-Anton, A.A., Zamora-Natera, J.F., Zarazua-Villasenor, P., Santacruz-Ruvalcaba, F., Sanchez-Hernandez, C.H., Alcantara, E.A., Torres-Moran, M., Velasco-Ramirez, A.P., & Hernandez-Herrera, R.M. (2023). Application of Seaweed Generates Changes in the Substrate and Stimulates the Growth of Tomato Plants. *Plants*, 12(7), 1520. https://doi.org/10.3390/plants12071520

Goncalves-Lopes, E.M., Reis, M.M., Frazo, L.A., Terra, L.E.M., Lopes, E.F., dos Santos, L.M., & Fernandes, L.A. (2021). Biochar increases enzyme activity and total microbial quality of soil grown with sugarcane. *Environmental Technology & Innovation*, 21, 101270. https://doi.org/10.1016/j.eti.2020.101270

Hamouda, R.A., Hussein, M.H., El-Naggar, N.E.A., Karim-Eldeen, M.A., Alamer, K.H., Saleh, M.A., Sharaf, E.M., & El-Azeem, R.M.A. (2022). Promoting effect of soluble polysaccharides extracted from *Ulva* spp. on *Zea mays* L. growth. *Molecules*, 27, 1394. https://doi.org/10.3390/molecules27041394

Higo, M., Azuma, M., Kamiyoshihara, Y., Kanda, A., Tatewaki, Y., & Isobe, K. (2020). Impact of Phosphorus Fertilization on Tomato Growth and Arbuscular Mycorrhizal Fungal Communities. *Microorganisms*, 8, 178. https://doi.org/10.3390/microorganisms8020178

Hou, Z., Tang, Y., Li, C., Lim, K. J., & Wang, Z. (2020). The additive effect of biochar amendment and simulated nitrogen deposition stimulates the plant height, photosynthesis and accumulation of NPK in pecan (*Carya illinoinensis*) seedlings. *AoB Plants*, 12, plaa035. https://doi.org/10.1093/aobpla/plaa035

Hu, W., Zhang, Y., Rong, X., Zhou, X., Fei, J., Peng, J., Luo, G. (2024). Biochar and organic fertilizer applications enhance soil functional microbial abundance and agroecosystem multifunctionality. *Biochar*, 6, 3 https://doi.org/10.1007/s42773-023-00296-w

Illera-Vives, M., Labandeira, S.S., Fernández-Labrada, M., & López-Mosquera, M.E. (2020). Agricultural uses of seaweed. In Sustainable Seaweed Technologies: Cultivation, Biorefinery, and Applications; Torres, M.D., Kraan, S., Dominguez, H., Eds.; Elsevier: Amsterdam, The Netherlands, pp. 591–612.

Jiang, Y., Wang, X., Zhao, Y., Zhang, C., Jin, Z., Shan, S., & Ping, L. (2021). Effects of Biochar Application on Enzyme Activities in Tea Garden Soi, 9, 728530. https://doi.org/10.3389/fbioe.2021.728530

Kasim, W.A., Saad-Allah, K.M., & Hamouda, M. (2016). Seed priming with extracts of two seaweeds alleviates the physiological and molecular impacts of salinity stress on radish (Raphanus sativus). *International Journal of Agriculture and Biology*, 2016, 18, 653–660. https://doi.org/10.17957/IJAB/15.0152

Khan, W., Rayirath, U., Subramanian, A., Jithesh, M., Rayorath, P.D., Hodges, M., Critchley, A., Craigie, J., Norrie, J., & Prithiviraj, B. (2009). Seaweed extracts as biostimulants of plant growth and development. *Journal of Plant Growth Regulation*, 28, 386-399 https://doi.org/10.1007/s00344-009-9103-x

Khan M.N., Lan Z., Sial T.A., Zhao Y., Haseeb A., Jianguo Z., Zhang A., & Hill R.L. (2019). Straw and biochar effects on soil properties and tomato seedling growth under different moisture levels. *Arch. Archives of Agronomy and Soil Science*, 65, 1704-1719, https://doi.org/10.1080/03650340.2019.1575510

Khan, S., Ismail, M., Ibrar, M., Haq, J.U., & Ali, Z. (2020). The Effect of Biochar on Soil Organic Matter, Total N in Soil and Plant, Nodules, Grain Yield and Biomass of Mung Bea. *Soil and Environment*, 39 (1), 87–94. https://doi.org/10.25252/se/2020/132088

Liao, N., Li Q., Zhang, W., Zhou, G., Ma, L., Min, W., Ye, J., & Hou,, Z. (2016). Effects of biochar on soil microbial community composition and activity in drip-irrigated desert soil. *The European Journal of Soil Biology*, 72, 27-34, https://doi.org/10.1016/j.ejsobi.2015.12.008

Ma, C., Song, W., Yang, J., Ren, C., Du, H., Tang, T., Qin, S., Liu, Z., & Cui, H. (2022). The role and mechanism of commercial macroalgae for soil conditioner and nutrient uptake catalyzer. *Plant Growth Regulation*, 97, 455–476. https://doi.org/10.1007/s10725-022-00819-8

Mahmoud, S.H., Salama, D.M., El-Tanahy, A.M.M., & Abd El-Samad, E.H. (2019). Utilization of seaweed (Sargassum vulgare) extract to enhance growth, yield and nutritional quality of red radish plants. *Annals of Agricultural Sciences*, 64, 167–175. https://doi.org/10.1016/j.aoas.2019.11.002

Messyasz, B., Pikosz, M., Schroeder, G., Łęska, B., & Fabrowska, J. (2015).Cultivation and Identification of Marine Algae. In Marine Algae Extracts. Processes, Products, and Applications; Kim, S., Chojnacka, K., Eds.; Wiley-VCH: Weinheim, Germany, 2015; Volume 1, pp. 17–40.

Michalak, I., & Messyasz, B. (2021). Concise review of Cladophora spp.: A macroalga of commercial interest. *Journal of Applied Phycology*, 33, 133–166. https://doi.org/10.1007/s10811-020-02211-3

Pathan, S.I., Zifcakova, L., Ceccherini, M.T., Pantani, O.L., Vetrovsky, T., & Baldrian, P. (2017). Seasonal variation and distribution of total and active microbial community of β-glucosidase encoding genes in coniferous forest soil. *Soil Biology Biochemistry*, 105, 71-80. https://doi.org/10.1016/j.soilbio.2016.11.003

Paz-Ferreiro, J., Fu, S., Méndez, A., & Gasco, G. (2013). Interactive Effects of Biochar and the Earthworm Pontoscolex Corethrurus on Plant Productivity and Soil Enzyme Activities. *Journal of Soils Sediments*, 14, 483–494. https://doi.org/10.1007/s11368-013-0806-z

Pikosz, M., & Messyasz, B. (2016). Characteristics of *Cladophora* and coexisting filamentous algae in relation to environmental factors in freshwater ecosystems in Poland. *Oceanological and Hydrobiological Studies*, 45, 202–215. https://doi.org/10.1515/ohs-2016-0019

Popko, M., Michalak, I., Wilk, R., Gramza, M., Chojnacka, K., & Gorecki, H. (2018). Effect of the New Plant Growth Biostimulants Based on Amino Acids on Yield and Grain Quality of Winter Wheat. *Molecules*, 23, 470. https://doi.org/10.3390/molecules23020470

Salim, B.B.M. (2016). Influence of biochar and seaweed extract applications on growth, yield and mineral composition of wheat (*Triticum aestivum* L.) under sandy soil conditions. *Annals of Agricultural Sciences*, 61, 257-265 https://doi.org/10.1016/j.aoas.2016.06.001

Song X., Razavi B.S., Ludwig B., Zamanian K., Zang H., Kuzyakov Y., Dippold M.A., & Gunina A. (2020). Combined biochar and nitrogen application stimulates enzyme activity and root plasticity. Science of the Total Environment, 735, 139393, https://doi.org/10.1016/j.scitotenv.2020.139393

Tabatabai MA (1994) Soil enzymes. In: Weaver RW, Angle S, Bottomley P, Bezdicek D, Smith S, Tabatabai A, Wollum A (eds) Methods of soil analysis. Part 2. Microbiological and biochemi - cal properties. Soil Science Society of America, Madison, pp 775–833

Tu C., Wei J., Guan F., Liu Y., Sun Y., & Luo Y. (20209. Biochar and Bacteria Inoculated Biochar Enhanced Cd and Cu Immobilization and Enzymatic Activity in a Polluted Soil. *Environment International*, 137, 105576. https://doi.org/10.1016/j.envint.2020.105576

Wang R., Fu W., Wang J., Zhu L., Wang L., & Wang J. (2019). Application of Rice Grain Husk Derived Biochar in Ameliorating Toxicity Impacts of Cu and Zn on Growth, Physiology and Enzymatic Functioning of Wheat Seedlings. Bull. *The Bulletin of Environmental Contamination and Toxicology*, 103 (4), 636–641. https://doi.org/10.1007/s00128-019-02705-y

Yin R., Deng H., Wang H.l., & Zhang B. (2014). Vegetation Type Affects Soil Enzyme Activities and Microbial Functional Diversity Following Re-vegetation of a Severely Eroded Red Soil in Sub-tropical China. *Catena,* 115, 96–103. https://doi.org/10.1016/j.catena.2013.11.015

Yıldız, M., & Özgen, M. (2004). The effect of media sucrose concentration on total phenolics content and adventitious shoot regeneration from sugar beet (*Beta vulgaris* L.) leaf and petiole explants. *Plant Cell, Tissue and Organ Culture*, 77,111-115.