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SYNERGISTIC EFFECTS OF BIOCHAR AND ARBUSCULAR MYCORRHIZAL FUNGI ON ENHANCING *ELYMUS ELYMOIDES* GROWTH IN SALINE COASTAL SOIL

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Abstract

The study aimed to investigate the effect of biochar (BC) application on soil properties, *Elymus elymoides* growth, and mycorrhizal fungi diversity. Soil samples were treated with different rates of BC (1%, 2%, and 5%) and inoculated with arbuscular mycorrhizal fungi (AMF) or without AMF. The results indicated that the application of BC at 2% and 5% rates significantly increased the soil pH by 4.3% and 5.8%, respectively. Moreover, the exchangeable potassium (K) levels showed a significant increase in soils treated with BC, i.e., K levels increasing by 66.7% and 211.1% at 2% and 5% BC application rates, respectively. Conversely, sodium (Na) levels in the soil decreased significantly with increasing BC application rates. The soil organic carbon (SOC) levels showed a significant increase in the treatment groups compared to the control group, with increases of 150%, 177.78%, and 222.17% in BC1, BC2, and BC5, respectively. The cation exchange capacity (CEC) values also showed a significant increase in BC1, BC2, and BC5, with increases of 14.81%, 29.63%, and 44.44%, respectively, compared to that in the control group. In terms of *Elymus elymoides* growth, BC2 showed the highest percent increase in plant height with 54.83%, reaching 27.33 cm, while BC1 and BC5 also had significant increases of 13.23% and 49.35%, respectively, reaching 20 cm and 26.33 cm. However, when AMF was added to the treatment groups, the plant growth decreased for all groups, with BC2 showing the highest percentage decrease of 28.07%, reaching 19.67 cm. The results of the mycorrhizal fungi diversity showed that biochar application had a significant effect on the growth and diversity of mycorrhizal fungi, with the BC and BC + AMF groups showing the most inhibitory effects. The addition of biochar had a negative effect on all types of mycorrhizal fungi, with the species of important genera such as *Rhizophagus*, *Glomus*, *Clarediogous*, and *Redochera* decreasing to 39.67%, 22.67%, 18%, and 16.33%, respectively. However, the addition of biochar and AMF together led to an increase in the abundance of *Rhizophagus* to 62.67%, *Glomus* to 67.67%, *Clarediogous* to 38.33%, and *Redochera* to 16.67%.

Key words: Salinity, Soil organic carbon, Enzyme activity, Soil microbial biomass, Plant community.

Introduction

Soil is essential for agricultural production and our food system, but unsustainable farming practices have diminished its capacity to support food production (Meyer *et al*., 2011). To address this challenge, effective supplements that can promote healthy and sustainable soil development are needed. Coastal salinization affects a significant portion of cultivable and irrigated land worldwide, making research on improving marine saline soils necessary to improve ecosystem service functions (Dubois, 2011). Carbon components, such as active organic carbon and particulate organic carbon, are more active than conventional indicators of total soil organic carbon and reflect dynamic changes in soil carbon storage and fertility (Major *et al*., 2010). Soil microorganisms play critical roles in soil decomposing, mineralization, organic matter decomposition, and plant nutrient delivery, and changes in the activity of specific functional microorganisms can reveal changes and differences in soil ecological function (Treseder & Allen, 2000). Biochar, which is produced from pyrolysis of biomass under oxygen-limited or anaerobic conditions, is a potential supplement to improve soil fertility (Barrow, 2012). Biochar mainly consists of carbon, with other trace elements, and has a porous honeycomb structure. It has been found to have a faster response rate than other physical and chemical indicators when applied to soil (Singh *et al*., 2010). Therefore, modifications in soil organic carbon composition and soil microbial diversity should be studied to understand the effects and mechanisms of biochar on improving soil fertility (Wildman & Derbyshire, 1991; Al-Wabel *et al*., 2018). Recent studies have demonstrated that combining biochar and Arbuscular Mycorrhizal Fungi (AMF) can be more effective in promoting plant growth in coastal saline soil than either component alone, particularly when high-quality AMF inoculants are applied (Ding *et al*., 2016). However, the underlying mechanism of this synergistic effect is still not fully understood, and further research is needed to explore the most effective combination of different materials with biochar to improve soil quality and plant growth in coastal saline environments. The present study aimed to investigate the effects of biochar and AMF on plant stress resistance and soil improvement. Specifically, we aimed to determine the impact of *Enteromorpha prolifera* biochar (BC) on soil organic carbon and microbial biomass in high-salinity coastal soils, while also examining the AMF population types, soil organic carbon-related enzymes, soil structure, and the role of AMF in organic carbon decomposition and *Elymus elymoides* growth in saline soil. Our study also aimed to identify the most effective combination of biochar and AMF that could enhance plant growth and

nutrient absorption, as well as improve soil quality in coastal saline environments. The novelty of our study lies in the investigation of the combined effects of biochar and AMF on improving soil quality, promoting plant growth of *Elymus elymoides*, and enhancing the living environment of AMF in coastal saline soil. By examining the specific mechanisms underlying this synergistic effect, our findings will provide valuable insights for developing sustainable agricultural practices and mitigating the impacts of climate change on food production in coastal areas.

Material and Methods

Soil collection and processing: Soil samples were collected (Petersen & Calvin, 1986) from a coastal area with high salinity in Karachi, Sindh Province, Pakistan (24° 56' 46.3848" N, 67° 0' 20.2140" E) using a multi-point mixed sampling method from a depth of 0-20 cm. The collected samples were delivered to the laboratory where they were air-dried, and debris such as stones and root remains was removed. The soil samples were then thoroughly mixed and passed through a 2 mm screen before being stored at room temperature for later use. Prior to investigating soil properties, one kg of each of soil samples was taken and air-dried, followed by its placement in a plastic container. This was done to compare the control and biochar-treated soils (Kim *et al*., 2016).

Enteromorpha prolifera collection and processing: *Enteromorpha prolifera* was collected from the Bahria University Karachi, Pakistan (24° 56' 46.3848" N, 67° 0' 20.2140" E) and used as a feedstock for biochar production. The characteristics of *Enteromorpha prolifera* and biochar are provided in (Table 1). The algae were sun-dried and cut into small pieces (7–10 cm). Subsequently, the material was pyrolyzed in an outdoor reactor at 450–500°C for 180 minutes. The resulting biochar was cooled, milled to pass a 2-mm sieve, and stored at room temperature. The moisture content of the biochar was 2.25% ± 0.5%.

Characterization of biochar: The pH of the *Enteromorpha prolifera* biochar was measured using a pH meter and found to be 8.7 with a biochar-to-water ratio of 1:2.5 (Page *et al*., 1983). The electrical conductivity (EC) of the biochar was 0.85-0.39 dS m^{-1} with a biochar-to-water ratio of 1:2.5 (Alghamdi *et al*., 2020). The biochar was fractionated into different particle sizes using dry sieving with 1, 0.5, and 0.1 mm sieves. The resulting particle size fractions were labeled as biochar sizes (BS) BS1, BS2, BS3, and BS4. The surface characteristics and porosity of the different biochar particle sizes were evaluated using the Brunauer Emmett–Teller (BET) technique with nitrogen (N_2) at 77K and a surface area and microporosity analyzer (Zhao *et al*., 2017). The total surface area and pore size of the four biochar particle sizes were as follows: BS1, BS2, BS3, and BS4 had a total surface area of 158.2, 176.2, 184.1, and 197.2 m^2 g^{-1}, and BS1, BS2, BS3, and BS4 had a pore size of 26.3, 27.4, 26.6, and 26.5 µm respectively.

Pot filling and treatment application rate: Each plastic container was filled with 5 kg of coastal saline soil (22 cm diameter × 24 cm depth). As per treatment plan, each pot received 100 g of non-mycorrhizal inoculum, 100 g of mycorrhizal inoculum and 100 g of non-mycorrhizal inoculum plus 100 g of *Enteromorpha prolifera* biochar. To achieve equilibrium, simulated contaminated the soil was incubated at room temperature for one month with 60% of the weight of deionized water holding capacity for each treatment, and the pots were randomly placed in four duplicates in the greenhouse. The characteristic of soils are provided in (Table 2).

Treatment plan: In a recent study, four different treatments were implemented to investigate their impact on plant growth and nutrient uptake. The first treatment, known as the control, received no treatment and was used as a baseline for comparison. The second treatment, T2, involved the inoculation of *Acaulospora scrobiculata* (As, BGC HK02A), a type of arbuscular mycorrhizal fungi (AMF), to facilitate nutrient uptake in the plants. The third treatment, T3, incorporated the use of *Enteromorpha prolifera* biochar amendment (BC), a type of soil amendment that has been shown to improve soil fertility and plant growth. Finally, the fourth treatment, T4, combined the application of biochar and AMF inoculation (AMF+BC) to investigate the potential synergistic effects of these two treatments on plant growth and nutrient uptake.

Seeds sowing: On May 10, 2021, three *Elymus elymoides* seeds were placed in each container, and after germination, they were trimmed. *Elymus elymoides* growth was kept under controlled temperature and natural light conditions during the growth period, and the soil was watered daily to keep it at 40-50% of its water storage capacity. No additional nutrients were supplied throughout the study.

Harvesting and data collection: Following six weeks of implantation, above-ground *Elymus elymoides* tissues were harvested and the activity of antioxidant enzymes in the newly harvested stalks was analyzed. The harvested tissue was washed twice with distilled water and once with tap water to eliminate any residual soil particles, and then dried in a fan-forced oven at 70°C for 72 hours to ensure complete dryness (Kim *et al*., 2016). The dried *Elymus elymoides* tissue was weighed, powdered, and stored in a desiccator prior to determining the total nutritional and elemental content. However, after 100 days of growth, plants from the *Elymus elymoides* were harvested, and soil samples were collected.

Sodium and potassium: Ion chromatography was used to determine the soils soluble Na^+ and K^+ ions. After being dried and sieved, a particular quantity of wind is weighed, and the soil is blended in a 1:2.5 soil-water ratio, agitated for three minutes, and then filtered immediately. After filtering with a microporous membrane, the filtrate was 0.22 m, and an ion chromatograph (ICS 3000 Di'an) was utilized for ion chromatography.

Table 1. Characteristics of *Enteromorpha prolifera* and resulting biochar.

Treatments	Proximate analysis (%) [a]						Nutrient contents (%) [b]					
	pH	Net surface area (m^2/g)	Moisture (%)	Volatile matter (%)	Fixed carbon (%)	Ash (%)	Carbon (%)	Nitrogen (%)	Hydrogen (%)	Oxygen (%)	H/C (%)	O/C (%)
Enteromorpha prolifera	6.4	0.66	4.2	66.0	8.2	21.3	32.6	0.6	4.8	39.2	1.7	0.8
Biochar	8.5	1.71	1.7	27.4	28.6	40.4	34.2	0.7	3.2	11.0	0.9	0.3

[a] = Wet weight percentage [b] = Dry weight percentage

Table 2. Characteristics of the study's soil.

Treatments	Composition (g kg^{-1} dry soil)							Texture		
	pH	ECe (mS cm^{-1})	Bulk density (g cm^{-3})	Total C (g kg^{-1})	Total N (g kg^{1})	Organic C (g kg^{-1})	Sand (%)	Silt (%)	Clay (%)
Control	7.4	2.61	1.38	29.3	0.28	5.1	94.3	3.0	2.6
Enteromorpha prolifera Biochar treated soil	8.0	19.73	1.25	40.1	0.47	26.5	88.2	4.0	7.5

AMF colonization: Subsamples of fresh roots from each pot were kept in FAA (37% formaldehyde, glacial acetic acid, % ethanol, 9:0.5:0.5, V/V/V) to determine the amount of AMF colonization. We weighed the total weight of fresh roots. The root and shoot tissues were heated to 105°C for 30 minutes to cease all enzymatic activity completely. After that, they were weighed after being consistently dried at 80°C. A microscope was used to determine the amount of root AMF colonization. The roots were decontaminated in 10% KOH (W/V) and stained with acid fuchsin (100). After harvesting plants, the percentage of root intersections containing arbuscular vesicles, or hyphae, was determined using Giovannetti and Mosse's gridline intersection method (Giovannetti & Mosse, 1980). The diversity among AMF was observed through DNA analysis from DNA in soil and humus and DNA in the root tip, where a MOBIO kit and kangyouwei kit were used for DNA concentration determinations, PCR amplification, library construction, and bioinformatics analysis.

Chlorophyll concentration and leaf N contents: A plant nutrition meter was used to test *Elymus elymoides* chlorophyll concentration and leaf N contents in every pot before harvest (TYS-3N, Zhejiang TOP Instrument Co., Ltd. China). An elemental analyzer was used to determine the amount of N in the plants (Flash 2000 EA-HT, Thermo Fisher Scientific, Inc., USA). Before beginning the harvesting process, the height of the plants was determined. The *Elymus elymoides* plants were harvested by severing the branches just below the surface of the earth, then gently segregating roots from the ground.

Growth attributes: Shoot dry, fresh weight; plant biomass was measured according to standard methods and spectrophotometry, and Kjeldahl measured leaf chlorophyll and N ion concentrations. Plant roots were subjected to measure the root length, diameter, projected area, volume, fresh weight, and surface area.

Enzymatic activities: Fresh tissue (0.2g) was used to measure enzyme activities. Samples were crushed using liquid nitrogen, and buffer Tris-HCl of 1 mL (0.05 M, pH 7.5) was included. The mixture obtained was centrifuged (at 4 ◦C and 13,000 PRESENT *g* value rpm) for 20 min, and the supernatant was used for enzyme activities measurement (Sudhakar *et al*., 2001). Catalase (CAT), peroxide (POD), malonaldehyde (MAD), and superoxidase (SOD) activity was assayed as per protocol given by Kar & Mishra (1976) using Tri's buffer with 5 mM H_2O_2 on an ice bath. The absorbance curve plotted at a wavelength of 240 nm and 245nm expressed based on mg protein/min.

Statistical analysis

The present study utilized standard statistical procedures to analyze the collected data, in accordance with established guidelines (Steel *et al*., 1997). Specifically, an analysis of variance (ANOVA) was conducted using the least significant difference test (LSD) to determine the statistical significance of the results. Multiple comparisons were made at a significance level of $p<0.05$ to ensure that the findings were reliable and valid. To facilitate these

statistical analyses, the software package Origin Pro 2021 (OriginLab Corporation, 2021) was employed. By adhering to these rigorous statistical procedures, the study was able to accurately evaluate the data and draw meaningful conclusions regarding the research questions at hand.

Results: The results showed that there was no significant change in pH in the soil treated with 1% BC compared to the control group. However, at 2% and 5% BC application rates, the pH increased significantly by 4.3% and 5.8%, respectively. The exchangeable K levels also showed a significant increase in the soil treated with BC compared to the control. At a 1% BC application rate, the K levels increased by 33.3%. This increase was even greater in soils treated with 2% and 5% BC, with K levels increasing by 66.7% and 211.1%, respectively. On the other hand, the Na levels in the soil decreased significantly with increasing BC application rates. The soil treated with 2% BC showed a significant decrease in Na levels by 18.0% compared to the control group. The greatest decrease in Na levels was observed in soils treated with 5% BC, which showed a 34.6% decrease in Na levels compared to the control. The ESP, which is the percentage of the soil occupied by exchangeable sodium, also showed a significant increase in soils treated with BC. The control group had an ESP of 12%, while soils treated with 1%, 2%, and 5% BC had ESP values of 88%, 71%, and 50%, respectively (Table 3).

The soil organic carbon (SOC) levels showed a significant increase in the treatment groups compared to the control group. BC1, BC2, and BC5 showed increases of 150%, 177.78%, and 222.17%, respectively, indicating a considerable increase in soil carbon sequestration. The calcium (Ca) levels showed no significant difference between the control and treatment groups. In contrast, the magnesium (Mg) levels showed a non-significant increase in BC1, BC2, and BC5, with increases of 62.5%, 125%, and 150%, respectively. However, cation exchange capacity (CEC) values showed a significant increase in BC1, BC2, and BC5, with increases of 14.81%, 29.63%, and 44.44%, respectively, compared to the control group (Fig. 1).

The control group without AMF had an average plant growth of 17.67 cm, while the control group with AMF had a slight decrease to 16.67 cm, a decrease of 5.98%. Among the treatment groups without AMF, BC2 showed the highest percentage increase in plant growth with 54.83%, reaching 27.33 cm, while BC1 and BC5 also had significant increases of 13.23% and 49.35%, respectively, reaching 20 cm and 26.33 cm. When AMF was added to the treatment groups, the plant growth decreased for all groups. BC2 showed the highest percentage decrease of 28.07%, reaching 19.67 cm, while BC1 and BC5 showed decreases of 5% and 10.05%, respectively, reaching 15.83 cm and 23.67 cm (Fig. 2).

Table 3. Variation in chemical properties of coastal soil dosed with 0.1% NaCl as affected by biochar (BC) treatment.

Attributes	Units	Control	BC 1%	BC 2%	BC 5%
pH	-	6.9 ± 0.02b*	6.9 ± 0.03b	7.2 ± 0.02a	7.3 ± 0.03a
K	Exchangeable	0.9 ± 0.01d	1.2 ± 0.0c	1.5 ± 0.0b	2.8 ± 0.04a
Na	(cmol kg^{-1})	13.3 ± 0.20a	10.8 ± 0.b	9.4 ± 0.7c	8.7 ± 0.3d
ESP	(%)	12 ± a	88 ± b	71 ± 7c	50 ± 2d

*Means in each column followed by the same letter are not significantly different ($p<0.05$) between control and treatments.
ESP = Electrostatic precipitation

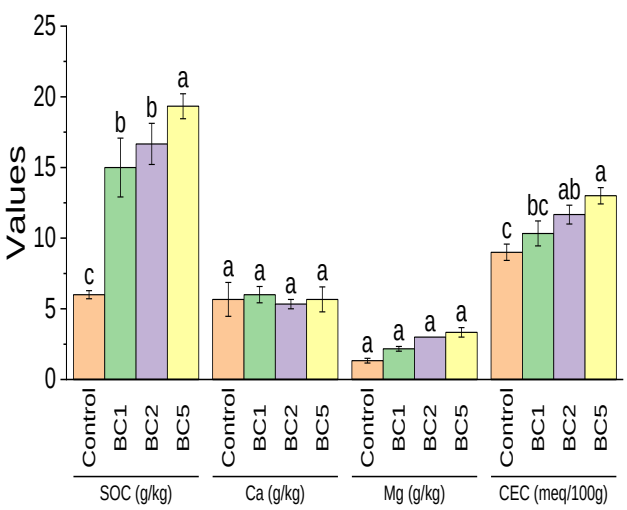

Fig. 1. Effect of different biochar additions on the changes of soil organic carbon (SOC), Ca^{2+}, Mg^{2+} and cation exchange capacity (CEC) contents of saline soil. Bars are means of three replicates ± SE. Different letters on the bars indicate significant differences at $p<0.05$ for change in soil organic carbon (SOC), Ca^{2+}, Mg^{2+} and cation exchange capacity (CEC) due to application of treatment.

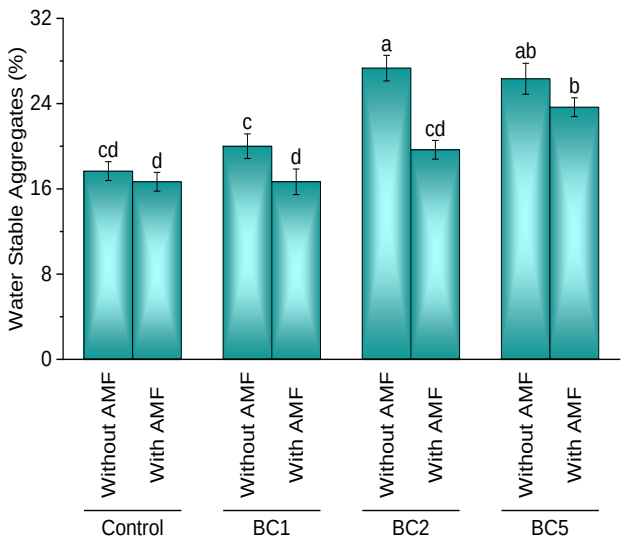

Fig 2. After treatment with biochar (BC), the water-stable aggregate proportion of coastal soil used for agriculture was changed. Bars are means of three replicates ± SE. Different letters on the bars indicate significant differences at $p < 0.05$ for change in water-stable aggregate due to application of treatment.

Table 4. The observed number of strains (NOS) and different mycorrhizal characteristics under biochar application (OUT= Operational taxonomic Unit).

Treatments	Observations			
	NOS	Number of OTU	OTU	Shanon effect
Control	37	9	38	31
BC	31	8	7	6
AMF	38	3.7	3.4	3.3
BC + AMF	31	1.13	1.09	1.02

Table 5. Effects treatments on growth parameters of *Elymus elymoides*.

Treatment	Plant height (cm)	Root length (cm)	Projected area (m^2)	Root surface area (m^2)	Root fresh weight (g)
Control	0.78 ± 0.01	69.58 ± 0.02	12.99 ± 0.05	5.38 ± 0.02	41.93 ± 0.02
BC	0.89 ± 0.023	102.39 ± 0.1	15.78 ± 0.02	6.11 ± 0.01	48.72 ± 0.02
AMF	0.81 ± 0.011	96.12 ± 0.01	15.07 ± 0.03	5.94 ± 0.02	44.83 ± 0.04
BC+AMF	0.85 ± 0.05	117.28 ± 0.02	18.78 ± 0.04	7.52 ± 0.06	46.91 ± 0.05

Values are means (3 replicates) ± SE (SE= Standard error)

Arbuscular mycorrhizal fungi inoculation: Table 4 presents the observations of the experiment on the number of strains (NOS) and different mycorrhizal characters under biochar application. The results showed that the control group had the highest number of strains (37) and operational taxonomic units (OTU) (9). In contrast, the BC (biochar) group had a lower NOS (31) and OTU (8) compared to the control. The AMF (arbuscular mycorrhizal fungi) group showed a significant decrease in the NOS (38) and OTU (3.7), indicating that biochar application inhibited the growth of mycorrhizal fungi. The BC + AMF group had the lowest NOS (31) and OTU (1.13) compared to all the other groups, indicating that the combination of biochar and AMF had a significant inhibitory effect on the growth of mycorrhizal fungi. Furthermore, the Shanon effect values of the groups showed that the control group had the highest value (31), indicating a higher diversity of mycorrhizal fungi. The BC, AMF, and BC + AMF groups showed significantly lower Shanon effect values (7, 3.4, and 1.09, respectively), indicating a lower diversity of mycorrhizal fungi. These results suggest that biochar application had a significant effect on the growth and diversity of mycorrhizal fungi, with the BC and BC + AMF groups showing the most inhibitory effects.

In the control group, *Rhizophagus* had the highest abundance at 81.67%, while *Glomus* and *Clarediogous* were also present at 16.33% and 87.67%, respectively. However, when AMF was added, the abundance of *Rhizophagus* decreased to 69%, while that of *Glomus* increased dramatically to 72.33%. *Clarediogous*, on the other hand, decreased to 28.33%. The addition of biochar had a negative effect on all types of mycorrhizal fungi, with *Rhizophagus*, *Glomus*, *Clarediogous*, and *Redochera* decreasing to 39.67%, 22.67%, 18%, and 16.33%, respectively. However, the addition of biochar and AMF together led to an increase in the abundance of *Rhizophagus* to 62.67%, *Glomus* to 67.67%, *Clarediogous* to 38.33%, and Redochera to 16.67%. Finally, *Diversispora* was present at 32.33% in the control group but decreased to 16% with the addition of AMF or biochar and increased slightly to 23% with the addition of biochar and AMF (Fig. 3).

The height of the plants increased by 14.1% in the BC treatment, 4.7% in the AMF treatment, and 9.0% in the BC+AMF treatment, compared to the control. The root length of the plants also showed a substantial increase of 47.1%, 38.0%, and 68.0% in the BC, AMF, and BC+AMF treatments, respectively, compared to the control. Furthermore, the projected area, root surface area, and root fresh weight of the plants all exhibited a significant increase in all treatments compared to the control. The projected area of the plants increased by 21.6% in the BC treatment, 16.2% in the AMF treatment, and 44.9% in the BC+AMF treatment. Additionally, the root surface area of the plants increased by 13.3% in the BC treatment, 10.5% in the AMF treatment, and 39.8% in the BC+AMF treatment. Moreover, the root fresh weight increased by 16.2%, 6.6%, and 11.9% in the BC, AMF, and BC+AMF treatments, respectively, compared to the control (Table 5).

In the case of root volume, it was found that all treatments showed an increase compared to the control treatment. The BC2 treatment showed the highest increase of 68.57% in root volume compared to the control. The control treatment showed a root diameter of 0.42667 mm, while all biochar treatments showed an increase in root diameter. The highest increase was found in the BC1 treatment, which showed an increase of 28.89% compared to the control (Fig. 4).

When compared to the control group, the plants treated with biochar (BC) alone showed a 150% increase in shoot dry weight, and a 7% increase in biomass, as well as a 63% increase in chlorophyll content, and a 15% increase in leaf nitrogen contents. Meanwhile, the group treated with arbuscular mycorrhizal fungi (AMF) alone showed a 178% increase in shoot dry weight, but a 6% decrease in biomass. The chlorophyll content and leaf nitrogen contents increased by 125% and 30%, respectively, compared to the control. Finally, the plants treated with a combination of biochar and AMF (BC+AMF) showed the most significant increase in shoot dry weight at 222%, along with a 7% increase in biomass, a 150% increase in chlorophyll content, and a 44% increase in leaf nitrogen contents, compared to the control. These results suggest that the combined use of biochar and AMF may have synergistic effects on the growth and development of *Elymus elymoides* plants (Fig. 5).

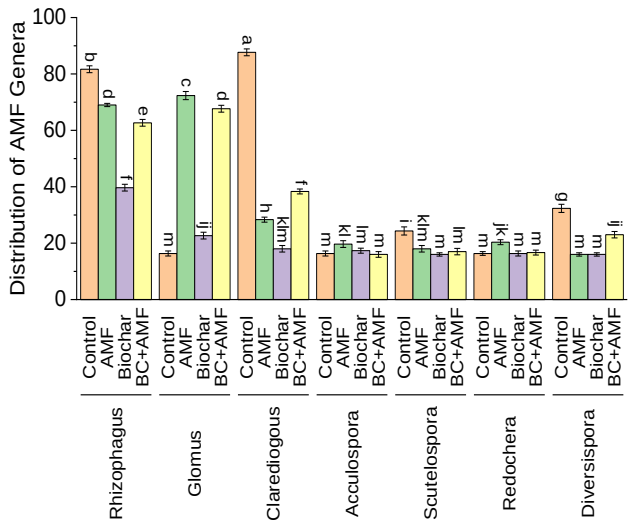

Fig. 3. Distribution of AMF genera identified in the roots of *Elymus elymoides*. Bars are means of three replicates ± SE. Different letters on the bars indicate significant differences at $p<0.05$ for change in distribution of AMF genera due to application of treatment.

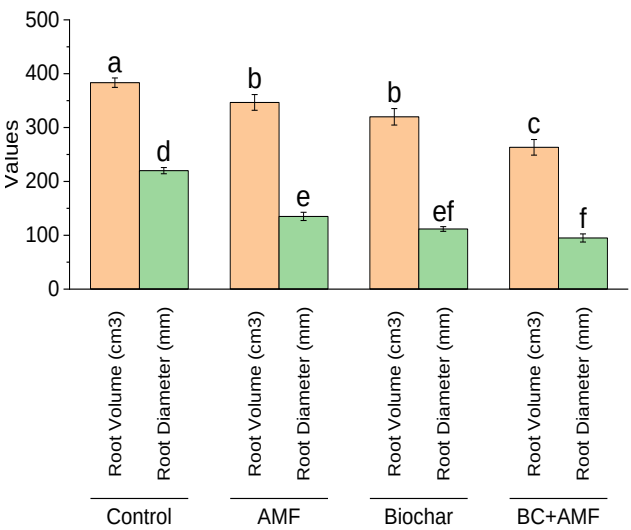

Fig. 4. Effect of biochar and AMF on root volume and root diameter of *Elymus elymoides*. For treatments: Control, un-inoculation AMF and no addition of biochar; BC, biochar addition; AMF, AMF inoculation; BC+AMF the co-application of biochar and AMF, represent the NaCl concentrations to which the plants were exposed. Bars are means of three replicates ± SE. Different letters on the bars indicate significant differences at p<0.05 for change in root volume and root diameter due to application of treatment.

The results for the different treatments are presented in terms of percentage increase or decrease compared to the control. The POD activity was decreased by 9.7% and 9.6% in the Biochar and BC+AMF treatments, respectively, while it was decreased by 9.6% in the AMF treatment. In contrast, the CAT activity was increased by 31.1%, 5.5%, and 32.7% in the Biochar, AMF, and BC+AMF treatments, respectively. Similarly, the SOD activity was increased by 49.4%, 38.6%, and 56.8% in the Biochar, AMF, and BC+AMF treatments, respectively, while it was decreased by 38.6% in the BC+AMF treatment. Moreover, the MDA content was decreased by 9.2% and 20% in the Biochar and BC+AMF treatments, respectively, while it was decreased by 8.7% in the AMF treatment. These results suggest that the application of biochar and BC+AMF may have a positive impact on antioxidant enzyme activity and lipid peroxidation in cucumber plants (Fig. 6).

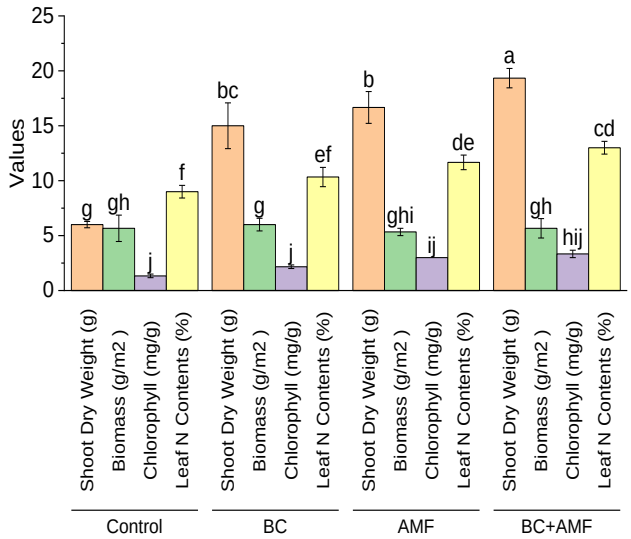

Fig. 5. Effect of biochar and AMF on shoot dry weight, biomass, chlorophyll and leaf nitrogen content of *Elymus elymoides*. For treatments: Control, un-inoculation AMF and no addition of biochar; biochar addition; AMF, AMF inoculation; BC+AMF the co-application of biochar and AMF, represent the NaCl concentrations to which the plants were exposed. Bars are means of three replicates ± SE. Different letters on the bars indicate significant differences at $p<0.05$ for change in shoot dry weight, biomass, chlorophyll and leaf nitrogen content due to application of treatment.

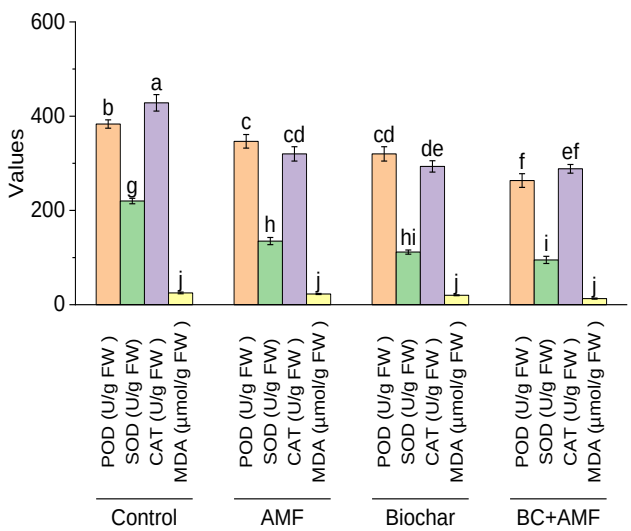

Fig. 6. Effect of biochar and AMF on SOD, POD and CAT activities of *Elymus elymoides*. For treatments: Control, un-inoculation AMF and no addition of biochar; BC, biochar addition; AMF, AMF inoculation; BC+AMF the co-application of biochar and AMF, represent the NaCl concentrations to which the plants were exposed. Different letters on the bars indicate significant differences at $p<0.05$ for change in SOD, POD and CAT activities due to application of treatment.

Discussion

Arbuscular Mycorrhizal Fungi (AMF) are a group of beneficial fungi that form a symbiotic relationship with the roots of plants. AMF can improve plant growth and development by enhancing nutrient uptake, particularly phosphorus, from the soil (Etesami *et al*., 2021). The fungi colonize the roots of the plants and form specialized structures called arbuscules, which facilitate nutrient exchange between the plant and the fungus (Wahid *et al*., 2020). The increased nutrient availability can lead to increased plant height, root length, and root surface area, ultimately resulting in higher plant productivity (Saboor *et al*., 2021a). Biochar is a type of charcoal that is produced by pyrolysis of organic material in a low-oxygen environment. Biochar has been shown to improve soil quality by increasing water-holding capacity, enhancing soil structure, and improving nutrient retention (Younis *et al*., 2020). Biochar can also provide a habitat for beneficial microorganisms, such as mycorrhizal fungi, which can further enhance plant growth. The enhanced soil quality and increased nutrient availability can lead to improved plant growth and development, including increased plant height, root length, and root surface area (Lehmann *et al*., 2011).

When plants have access to more nitrogen, they are able to produce more chlorophyll, which is the pigment that gives leaves their green color. Chlorophyll is essential for photosynthesis, the process by which plants convert sunlight into energy (Lawlor, 2001). Therefore, increased nitrogen uptake can lead to increased chlorophyll production, which can improve a plant's ability to photosynthesize and grow (Lawlor, 2001). Similar kind of results were also observed in current study where AMF and BC when applied in combination played a vital role in improvement of chlorophyll and nitrogen contents.

Furthermore, CAT (catalase) is an important enzyme that plays a vital role in the antioxidant defense system of plants, helping to detoxify reactive oxygen species (ROS) that can cause harm to cellular components (Gondim *et al*., 2012). Arbuscular mycorrhizal fungi (AMF) have been shown to have a positive effect on plant species, by regulating the CAT and other enzymes (Kullu *et al*., 2020). AMF establish a symbiotic relationship with plants, where the fungi colonize the roots and form a network of hyphae that extends into the surrounding soil. This network can increase the surface area available for nutrient uptake by the plant, allowing for greater uptake of essential nutrients such as nitrogen and phosphorus (Saboor *et al*., 2021b). Moreover, AMF can produce compounds that stimulate plant growth and enhance plant tolerance to environmental stressors (Saboor *et al*., 2021b). Studies have shown that AMF can regulate the activity of catalase in *Elymus elymoides*, which may contribute to the plant's ability to cope with oxidative stress (Khalvati *et al*., 2010, Alam *et al*., 2019). In addition, AMF may enhance the efficiency of nutrient uptake by the plant, which can reduce the stress negative impact (Saboor *et al*., 2021c).

Incorporating biochar into soil resulted in an increase in organic carbon concentration from 1 to 2.5%, or 10 grams of carbon per kg of dry soil. This was accompanied by a slight decline in bulk density, which went from 1.3 to 1.2 g cm^{-3}. In carbon-deficient soils like the one used in this study, adding biochar to soil can potentially increase organic carbon concentrations effectively (van Zwieten *et al*., 2010). Biochar not only encourages the growth of organic carbon content in the soil but also interacts with AMF through plant roots. The organic carbon content of saline soil in the mixed group was much greater than that of the non-saline soil, approximately an increase (Lilleskov *et al*., 2002). Furthermore, the addition of biochar to the soil can also contribute to the enhancement of soil quality and plant growth. Biochar is a carbon-rich material produced from the pyrolysis of organic waste materials such as agricultural residues, wood chips, and other biomass sources. When added to soil, biochar can increase soil organic carbon content, improve soil fertility, and enhance soil water holding capacity (Thies & Rillig, 2009).

When used in combination, biochar and AMF can have synergistic effects on plant growth in saline soils. Biochar can provide a porous habitat for AMF to colonize, and the carbon in biochar can act as a food source for the fungi. AMF, in turn, can help the plant to better access the nutrients and water in the soil, which can further enhance the benefits of biochar (Lehmann *et al*., 2011). In terms of their effects on plant growth attributes, both biochar and AMF can improve chlorophyll content and growth parameters such as shoot and root biomass (Amoakwah *et al*., 2022, Sun *et al*., 2022). Biochar can also help to reduce oxidative stress in plants by increasing the availability of antioxidants in the soil, while AMF can reduce the production of reactive oxygen species (ROS) in plant cells (Alam *et al*., 2019, Naveed *et al*., 2020). Overall, the combination of biochar and AMF can provide a multi-pronged approach to improving plant growth in saline soils. By reducing oxidative stress and improving nutrient uptake, these treatments can help to enhance the growth attributes of *Elymus elymoides* and other crops in challenging environments.

Conclusion

In conclusion, the application of BC at 2% and 5% rates could improve soil properties, increase plant growth, and affect mycorrhizal fungi diversity. However, the addition of AMF could reduce the positive effect of BC on plant growth, while the combination of biochar and AMF could have an inhibitory effect on the growth and diversity of mycorrhizal fungi. Further studies are needed to determine the long-term effects of biochar application and its interactions with AMF on soil health and plant growth.

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