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Original article

Biochar and Arbuscular mycorrhizal fungi mediated enhanced drought tolerance in Okra (*Abelmoschus esculentus*) plant growth, root morphological traits and physiological properties

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ABSTRACT

Drought is a major abiotic factor limiting plant growth and crop production. There is limited information on effect of interaction between biochar and Arbuscular mycorrhizal fungi (AMF) on okra growth, root morphological traits and soil enzyme activities under drought stress. We studied the influence of biochar and AMF on the growth of Okra (*Abelmoschus esculentus*) in pot experiments in a net house under drought condition. The results showed that the biochar treatment significantly increased plant growth (the plant height by 14.2%, root dry weight by 30.0%) and root morphological traits (projected area by 22.3% and root diameter by 22.7%) under drought stress. In drought stress, biochar treatment significantly enhanced the chlorophyll 'a' content by 32.7%, the AMF spore number by 22.8% and the microbial biomass as compared to the control. Plant growth parameters such as plant height, shoot and root dry weights significantly increased by AMF alone, by 16.6%, 21.0% and 40.0% respectively under drought condition. Other plant biometrics viz: the total root length, the root volume, the projected area and root diameter improved significantly with the application of AMF alone by 38.3%, 60.0%,16.8% and 15.9% respectively as compared with control. Compared to the control, AMF treatment alone significantly enhanced the total chlorophyll content by 36.6%, the AMF spore number by 39.0% and the microbial biomass by 29.0% under drought condition. However, the highest values of plant growth parameters (plant height, shoot dry weight, root dry weight) and root morphological traits (the total root length, root volume, projected area, root surface area) were observed in the combined treatment of biochar and AMF treatment viz: 31.9%, 34.2%, 60.0% and 68.6%, 66.6%, 45.5%, 41.8%, respectively compared to the control under drought stress. The nitrogen content, total chlorophyll content and microbial biomass increased over un-inoculated control. The soil enzymes; alkaline phosphatase, dehydrogenase and fluorescein diacetate enzyme activities significantly increased in the combined treatment by 55.8%, 68.7% and 69.5%, respectively as compared to the control under drought stress. We conclude that biochar and AMF together is potentially beneficial for cultivation of okra in drought stress conditions.

1. Introduction

Drought stress is a major environmental stress, causing reduction of biological function in many crops (Pereira et al., 2006; Golldack et al., 2014; Hussain et al., 2018; Shehzad et al. 2021). Drought stress negatively impacts seed germination (Kaya et al., 2006; Farooq et al., 2009). Under drought condition the germination rate was reduced in chickpea (Awari and Mate 2015). Several studies have observed the negative effects of drought stress on

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plant growth of maize (Anjum et al., 2017; Dar et al, 2021), chickpea (Samarahet al., 2009; Pushpavalli et al., 2015), rice (Hussain et al., 2016; Wang et al., 2016), tomato (Starck et al., 2000) and soybean (Kobraee et al., 2011; Li et al., 2013; Maleki et al.,2013; Sheteiwy et al., 2021). Maes et al. (2009) reported that *Jatropha curcas* under different water regimes identified anatomical and morphological changes with an increase in adaxial stomatal ratio in response to drought. Drought negatively affects yield in different crops such as okra (Mueller et al., 2019; Chaturvedi et al. 2019; Ranawake et al., 2012; Haider et al. 2021), faba bean (Li et al., 2018) and chickpea (Pushpavalli et al., 2015). The impact of drought stress was studied on plant growth and yield of okra plant by Abdulrahman and Nadir (2018).

Physiological and biochemical functions of plants such as turgor (Chowdhury et al. 2016), mesophyll conductance (Zhou et al., 2013; Zhou et al., 2014), photosynthesis (Christophe et al., 2011; Mak et al., 2014; Osakabe et al., 2014; Lyu et al., 2016; Asha et al., 2021), transpiration rate (Asha et al., 2021), and relative water content (Saccardy et al., 1998; Sanchez-Blanco et al., 2006), reduced under drought stress. Soltys-Kalina et al. (2016) showed that the 3-week drought treatment decreased the leaf water content of potato cultivars. Drought stress significantly reduced chlorophyll *a*, *b* and total pigments in wheat and pumpkin (Al.-Ayed 1998; Sawhney and Singh 2002). Farooq et al. (2009) reported drought stress reduced leaf water potential and turgor pressure, stomatal closure, and decreased cell growth. Water stress reduced the amounts of starch and total sugar (Sawhney and Singh 2002). Begum et al. (2019) indicated that drought stress decreased plant height, chlorophyll and carotenoid content in Maize (Zea mays). Chaturvedi et al. (2019) observed that the significant reduction in relative water content and membrane stability index along with reduced leaf photosynthetic rate explains the possible membrane damage in okra affecting photosynthetic efficiency in drought condition. Several studies have reported that drought stress decreases the protein concentration of plants (Schwanz et al., 1996; Heckathorn et al., 1997).

Studies have shown that drought stress reduced uptake of plant nutrients (Razi and Sen, 1996; Christophe et al., 2011; Sardans and Peñuelas 2012; Rouphael et al., 2012; Heckathorn et al., 2014). soil nutrients (Cramer et al., 2009; Waraich et al., 2011; Fierer and Schimel, 2002) and soil enzyme activities (Sanaullah et al., 2011). Bista et al (2018) indicated that drought reduced N and P content, indicating that it reduced nutrient acquisition. Drought stress decreases the concentration of nitrogen and phosphorus in plant tissue. Ge et al. (2012) demonstrated that drought stress induced sharp decreases in total K and P uptake of maize organs. Sardans and Peñuelas (2005) reported that water stress decreased urease, protease activity, phosphatase activity and β-glycosidase activity in soil. Geng et al. (2015) reported that drought strongly affect soil respiration, soil microbial activity and fungal properties.

Biochar is a carbon-enriched biomaterial prepared through a process called pyrolysis (McGlashan et al. 2012). Lehmann et al. (2006) studied biochar benefits on reducing emissions and sequestering of greenhouse gases, impacting soil quality. Many have reported application of biochar, enhancing soil fertility, carbon sequestration and bio-energy production (Fiaz et al., 2014; Ok et al., 2015; Rizwan et al., 2016; Jabborova et al., 2020a). Biochar application to soils increased crop production due to the improvement of soil physicochemical and biological properties (Ahmad et al. 2014). Biochar enhanced soil structure, water holding capacity and surface area under drought condition (Andrenelli et al. 2016; Bamminger et al. 2016; Lim et al. 2016; (Yaseen, 2021)). Biochar positive effect on the plant growth (Kammann et al. 2011; Artiola et al. 2012; Mulcahy et al. 2013), yield (Akhtar et al. 2014) plant nutrients (Usman et al., 2016) and plant physiological properties (Haider et al. 2015; Lyu et al. 2016; Xiao et al. 2016) were studied in different plants under drought stress. Several studies have reported that biochar application increased plant biomass and nutrient uptake under water stress (Kammann et al. 2011; Akhtar et al. 2014; Haider et al. 2015; Kubar et al., 2021). The biochar application increased leaf quality rate and growth of tomato over the control (Githinji 2014; Vaccari et al. 2015). Addition of biochar significantly enhanced the photosynthetic rate, chlorophyll contents, stomatal conductance, relative water contents and water use efficiency in tomato leaves under drought stress (Akhtar et al., 2014). Batool et al. (2015) demonstrated that biochar increased the water use efficiency and photosynthesis of okra in drought stress condition.

Arbuscular mycorrhizal fungi (AMF) are important groups of soil microbes in symbiotic relationship with plant roots (Brundrett and Tedersoo, 2018). AMF are major component of rhizosphere microflora in natural ecosystems and play a significant role in ecosystems through nutrient cycling (Heflish et al., 2021) . AMF helps to improve higher branching of plant root system, plant growth and productivity of several field crops (Cavagnaro et al., 2006; Nunes et al., 2010; Alizadeh et al., 2011; Abd El-Aal et al., 2021). Abdel Latef (2011) reported that the plants inoculated with AMF increased plant photosynthesis, plant enzyme activities such as superoxide dismutase, catalase, peroxidase and ascorbate peroxidase. Several studies have reported that AMF improve the growth, plant nutrient and water uptake of host plants under drought stress (Gholamhoseini et al., 2013; Baum et al., 2015; Zhao et al., 2015a, 2015b; Bowles et al., 2018). Augé (2001) reported that AMF enhance plant performance, change the plant–water relationship, and improve plant productivity in drought condition. The AMF increased water use efficiency and stomatal conductance (Birhane et al., 2012; Ruiz-Lozano and Aroca, 2010). Augé et al. (2015) demonstrated that AMF improved water use efficiency and stomatal conductance in drought stress. Subramanian and Charest (1999) reported that the AMF enhanced the nitrogen availability of host plant in drought stress. Pedranzani et al. (2016) observed that AMF improved plant physiological properties such as antioxidant enzyme activity and jasmonate synthesis of *Digitaria eriantha* under drought stress. Biochar amendment and AMF inoculation improved plant growth, plant nutrition, photosynthetic rate and stomatal morphology under drought stress (Hashem et al., 2019).

Medicinal plants have been used in most parts of the world and has become of increasing interest in recent times for the use of various plants as sources of molecules having medicinal properties (Egamberdieva and Jabborova, 2018; Jabborova et al., 2019; Jabborova et al. 2020b; Mamarasulov et al. 2020;Jabborova et al., 2021a; Jabborova et al., 2021b; Jabborova et al., 2021c). Okra (*Abelmoschus esculentus* L.) is a vegetable and herbal crop; possess nutraceutical and therapeutic properties, owing to the presence of various important bioactive compounds and their associated bioactivities (Elkhalifa et al., 2021). A little is known about the combined effect of biochar and AMF on plant growth and physiological properties in drought stresses. The present study was conducted to evaluate the prospective effect of biochar and AMF application on okra plant growth, root morphological traits, physiological properties, microbial biomass, the number of AMF spores and soil enzymatic activities under drought condition.

2. Materials and methods

2.1. Soil, biochar, AMF and seed

Field soil collected from Indian Agricultural Research Institute was used for the experiment. The biochar used in the study was produced at 400–500 °C from woody biomass (Amazon online

shop, New Delhi, India), with a particle size of less than 2 mm. Variety Pusa A-4 seed was procured from Division of Vegetable Science, and AMF from the Division of Microbiology, IARI, New Delhi, India respectively.

2.2. Experimental design

The impact of biochar and AFM on the growth of Okra (*Abelmoschus esculentus*) was studied in pot experiments in a net house at Division of Microbiology, IARI, New Delhi, India. All the experiments were carried out in a randomized block design with three replications. Experimental treatments included: T1 control (soil without biochar), T2 biochar alone, T3 AFM alone and T4 combined biochar + AMF. Seed was cultivated into plastic pots (20 cm diameter, 20 cm depth) containing 5.0 kg of soil. During the 40 days of plant growth, drought conditions (50% of the field capacity) were maintained. After forty days plants were harvest and plant height, shoot and dry root weights were measured.

2.3. Measurement of root morphological traits of okra

The roots of okra were washed carefully with water. The whole root system was spread out and analyzed using a scanning system (Expression 4990, Epson, CA) with a blue board as a background. Digital images of the root system were analyzed using Win RHIZO software (Régent Instruments, Québec, Canada). The total root length, the root surface area, the root volume, the projected area and the root diameter were evaluated.

2.4. Organic elemental analysis

C and N were determined by Elemental Analyzer (CHNS) Eurovictor. For this purpose, 0.5 mg of each sample was placed in tin capsules and completely oxidized, at 950℃, to their elemental gases. The resultant combustion products were mechanically homogenized in a gas control zone and separated in a gas chromatographic column. Finally, eluted gases were conveyed to a thermal conductivity detector and amounts of N and C obtained.

2.5. Physiological parameter measurement

SPAD values were analyzed using the leaves from the okra plants using a SPAD-502 m (Konica-Minolta, Japan). SPAD measurements were made as estimates of chlorophyll content.

2.6. Analysis of AMF spores from soil

The AMF spores were extracted from 10 g soil samples using wet sieving and decanting method. Soil sample was put over a series of soil sieves arranged in descending order of sieve sizes. The clean spores were mesh sieved and washed several times with distilled water before being transferred into water in a clean Petridish. The AMF spores were counted under a stereomicroscope (Dare et al. 2013).

2.7. Analysis of soil microbial biomass determination

The method to measure biomass C was as given by Vance et al. (1987). Three of six 17.5 g replicates of each soil sample were fumigated with purified $CHCl_3$, for 24 h. After removal of the $CHCl_3$, the C was extracted from fumigated and unfumigated samples with 0.5 M K_2SO_4, for 1 h on an end-over-end shaker. The fumigated and unfumigated samples were filtered sequentially through filter paper (Whatman). The obtained supernatant liquid was measured at 280 nm.

2.8. Analysis of soil enzymes

The alkaline phosphatase activity was assayed by the method given by Tabatabai and Bremner (1969). For each soil, two sets of 1 g soil were placed in conical flasks. One set was used as the control. Then 0.2 mL toluene and 4 mL of MUB (modified universal buffer) (pH 11) were added and 1 mL of p-nitrophenyl phosphate solution was added to the other set of samples. These were incubated at 37 ^{O}C for 1 hr. Calcium chloride (1 mL of 0.5 M) and 4 mL of 0.5 M NaOH were added after incubation. Flasks were swirled for a few seconds and 1 mL of p-nitrophenyl phosphate solution was added to the remaining set of samples. All suspensions were filtered through Whatman No. 1 filter paper quickly and the yellow colour intensity was measured at 440 nm wavelength.

The fluorescein diacetate hydrolytic activity was determined following the method of Green et al. (2006). 0.5 mg soil was incubated with 25 mL of sodium phosphate (0.06 M; pH 7.6). 0.25 mL of 4.9 mM FDA substrate solution was added to all assay vials. All vials were mixed and incubated in a water bath at 37 °C for 2 h. Then soil suspension was centrifuged at 8000 rpm for 5 min. The clear supernatant was measured at 490 nm against a reagent blank solution in a spectrophotometer.

Dehydrogenase activity was determined using the method described by Casida et al. (1964). Fresh homogenized soil samples (5 g) were placed in test tubes with 5 mL substrate (3% v/w TTC). The tubes were incubated at 25 °C for 24 h. A blank sample was similarly prepared with 1 mL of a 3% TTC solution. After incubation, the samples were centrifuged at 4500 rpm for 10 min. The supernatant liquid was discarded. The TPF formed was extracted with methanol. 5 mL of methanol was added to each of the tubes and vigorously shaken for a few minutes. The operation was repeated twice (10 mL of methanol was used for extraction). Again the tubes were centrifuged. The obtained supernatant liquid was poured into a clean tube, and the absorbance of the solution was measured at 485 nm.

2.9. Statistical analyses

Experimental data were analyzed with the StatView Software using ANOVA. The significance of the effect of treatment was determined by the magnitude of the F value ($P < 0.05 < 0.001$).

3. Results

The result presented in Table 1 indicated the biochar treatment significantly increased the plant height by 14.2% and root dry weight by 30.0% as compared to control under drought stress. The okra plant height, shoot dry weight and root dry weight significantly enhanced under the treatment involving the AMF alone (Table 1). AMF treatment significantly increased the plant height by 16.6%, shoot dry weight by 21.0% and root dry weight by 40.0% compared to the control. In drought condition, when the combination of biochar and AMF treatment were applied, the plant

Table 1
Effect of drought stress on plant height, shoot dry weight and root dry weight in okra.

Treatments	Plant height (cm)	Shoot dry weight (g)	Root dry weight (g)
Control	21.00 ± 0.80	0.76 ± 0.01	0.10 ± 0.01
Biochar	24.00 ± 0.85*	0.85 ± 0.01	0.13 ± 0.01*
AMF	24.50 ± 0.50*	0.92 ± 0.01*	0.14 ± 0.01*
Biochar + AMF	27.76 ± 0.15*	1.02 ± 0.01*	0.16 ± 0.01*

Data are means of three replicates (n = 3), * asterisk differed significantly at $P < 0.05$*

Table 2
Effect of drought stress on root morphological traits in okra.

Treatments	Total root length (cm)	Projected area (cm^2)	Root surface area (cm^2)	Root volume (cm^3)	Root diameter (mm)
Control	69.58 ± 4.13	12.99 ± 0.76	5.38 ± 0.57	0.15 ± 0.01	0.44 ± 0.01
Biochar	102.39 ± 1.80*	15.78 ± 0.90*	6.11 ± 0.60	0.21 ± 0.01**	0.54 ± 0.01*
AMF	96.12 ± 4.11*	15.07 ± 0.36	5.94 ± 0.09	0.24 ± 0.01*	0.51 ± 0.01*
Biochar + AMF	117.28 ± 5.49**	18.78 ± 2.13**	7.52 ± 1.17**	0.25 ± 0.01**	0.56 ± 0.02**

Data are means of three replicates (n = 3), * asterisk differed significantly at P < 0.05*, P < 0.01**

height improved by 31.9%, shoot dry weight by 34.2% and root dry weight by 60.0% compared to the control respectively.

In Table 2, mean data regarding the root morphological traits as affected by the biochar and AMF application in drought condition is presented. The root morphological traits indicated that root parameters significantly increased the total root length, the root surface area, the projected area, the root diameter and the root volume by biochar alone, AMF alone and combined with biochar with AMF treatment under drought stress. Compared to the control, biochar alone treatment significantly enhanced the projected area by 22.3% and the root diameter by 22.7% under drought stress. The total root length and root volume sharply increased by biochar alone, which significantly increased by 47.3% and 40.0% respectively than the control. The projected area and root diameter was improved with the application of AMF by 16.8% and 15.9% as compared with control, respectively. Under drought stress, the AMF alone significantly enhanced the total root length by 38.1% and the root volume by 60.0%. The highest values of total root length (68.6%) and root volume (66.6%) were observed in the treatment of biochar and AMF combination as compared to control and individuals under drought stress. Similarly, significant increase in the projected area (45.5%), root surface area (41.8%), and root diameter (27.2%) were also observed.

As shown in Fig. 1, the biochar alone and AMF alone treatments marginally increased the nitrogen content in okra leaf under drought condition. The AMF alone application increased the nitrogen content by 9.0% compared to the control in drought stress. The nitrogen content was highest in the combined treatment of biochar and AMF. Under drought stress, combination of biochar and AMF treatment significantly enhanced the nitrogen content by 49.5% over the control.

Results showed that combination of biochar and AMF resulted in the highest carbon content compared to all treatment (Fig. 2). Under drought condition, biochar alone and AMF alone treatments gradually increased the carbon content as compared to control. Compared to the control, combination of biochar and AMF treatment significantly increased the carbon content by 10.4% under drought stress.

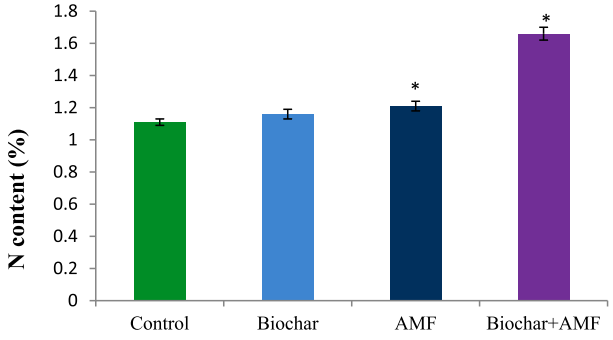

Fig. 1. Effect of drought stress on the nitrogen content of leaf in okra. Data are means of three replicates (n = 3), * asterisk differed significantly at P < 0.05*.

Data in Fig. 3 indicated that under drought stress, biochar alone and AMF treatments significantly increased total chlorophyll a content compared to the control. In drought stress, biochar treatment significantly enhanced the chlorophyll *a* content by 32.7% than the control and AMF alone significantly enhanced total chlorophyll content by 36.6% in drought condition. The highest values of total chlorophyll content was observed in the combined treatment of biochar and AMF recording a significant increase of 39.9% compared to the control under drought stress.

AMF spore number (per g of soil) increased in the treatment of biochar alone (Fig. 4). Biochar alone treatment significantly increased the AMF spores number by 22.8%. Under drought stress, AMF alone and combined with biochar and AMF treatments were more effective in increasing the AMF spores number in soil (Fig. 4). Compared to the control, the AMF spores in soil increased by 39.0% in AMF alone treatment. However, in the treatment where biochar and AMF were combined it significantly enhanced the AMF spores number by 51.5% compared to the control under water stress.

In water stress condition, biochar alone and AMF had significant impacts on the microbial biomass in soil increased most under 18.5% and 29.0% as compared the control (Fig. 5). Under drought stress, the microbial biomass reached a maximum in biochar and AMF combined treatment compared with all treatments. This treatment significantly increased the microbial biomass by 39.3% in soil than the control under drought stress.

The effect of biochar alone, the AMF alone and combined with biochar and AMF treatments on soil enzymes activities are given in Table 3. Compared to the control, biochar alone significantly influenced the alkaline phosphatase, the dehydrogenase and fluorescein diacetate enzyme activities in soil under drought condition. The dehydrogenase and fluorescein diacetate activities increased by 36.4% and 42.7% respectively when soil was amended by biochar alone as compared to the control in drought stress. Similarly, the alkaline phosphatase activity of the biochar alone and the AMF alone treatments significantly enhanced by 35.8% and 42.0% than the control. Under water stress, the dehydrogenase and fluorescein diacetate enzyme activity increased by 43.7% and 46.5% in AMF alone treatment as compared to the control. In drought condition, interaction between biochar and AMF significantly increased the alkaline phosphatase, the dehydrogenase and fluorescein diacetate enzyme activities in soil and were found much greater as compared to all other treatments. The increase was 55.8% (phosphatase), 68.7% (dehydrogenase enzyme) and 69.5% (fluorescein diacetate) in soil as compared to the control.

4. Discussion

We have studied the influence of biochar and AFM on the growth of okra (*Abelmoschus esculentus*) under drought stress condition. Drought stress reduces plant height, shoot dry weight and root dry weight. Drought stress and salinity stress decreased the germination rate and plant growth in various crops (Farooq et al., 2009; Awari and Mate 2015; Hussain et al., 2016; Egamberdieva and Jabborova, 2013; Egamberdieva et al., 2016; Egamberdieva

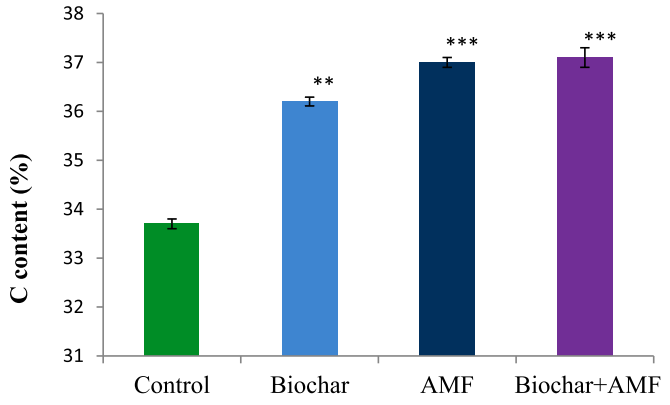

Fig. 2. Effect of drought stress on the carbon content of leaf in okra. Data are means of three replicates (n = 3), * asterisk differed significantly at P < 0.05*,.

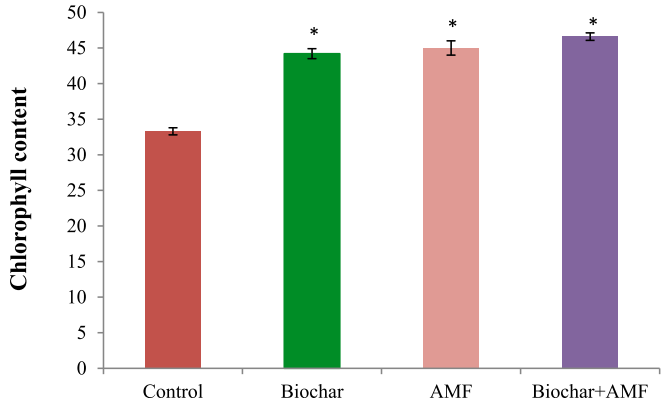

Fig. 3. Effect of drought stress on the chlorophyll content of leaf in okra. Data are means of three replicates (n = 3), * asterisk differed significantly at P < 0.05*.

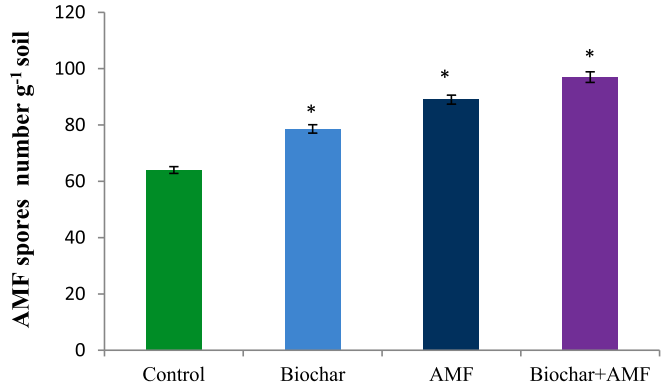

Fig. 4. Effect of drought stress on the AMF spore numbers in soil. Data are means of three replicates (n = 3), * asterisk differed significantly at P < 0.05*

et al., 2017; Jabborova et al., 2020c; Sheteiwy et al., 2021; Ijaz et al., 2021). Similarly, Mueller et al. (2019) reported that okra growth and the total dry weight of okra was reduced by drought stress. Water stress reduced the plant growth parameter such as shoot fresh weight, dry weight, leaf number, leaf area, plant height and stem diameter in okra (Kusvuran 2012). Similarly, drought stress reducing plant height of *Brassica napus* has been reported by Zhao et al. (2006). Raza et al. (2012) observed a decrease in plant height in wheat by water stress.

In the present study, biochar significantly increased plant height, root dry weight, total root length, projected area and the root diameter as compared to control under drought stress. Numerous scientists reported that biochar improved plant growth, development and yield in various plants under stress (Kammann et al. 2011; Artiola et al. 2012; Mulcahy et al. 2013; Jabborova et al., 2021d). Similarly, Hashem et al. (2019) reported that biochar enhanced shoot length, root length, leaf area, number of primary branches, plant number of secondary branches in chickpea under drought stress.

Haider et al. (2015) observed a positive effect of biochar amendment on stem and leaf dry weight in maize. This finding is consistent with the report of de MeloCarvalho et al. (2013) who observed biochar amendment increased the leaf area index, biomass and yield in rice under water stress.

According to Batool et al. (2015) biochar application increased the leaf area, plant height in okra (*Abelmoschus esculentus* L.). Olmo et al. (2014) reported that biochar increased biomass of field grown wheat under semiarid Mediterranean conditions.

Studies have shown that the AMF significantly increased improved plant height, shoot dry weight, total root length, root volume and root diameter under drought stress. Numerous studies have reported that AMF improve the growth and water uptake of host plants under drought stress (Gholamhoseini et al., 2013; Baum et al., 2015; Benhiba et al., 2015; Zhao et al., 2015a, 2015b; Chitarra et al., 2016; Quiroga et al., 2017; Bowles et al., 2018). This finding is consistent with the report of Hashem et al. (2018) who observed an increase in chickpea shoot length, root length, leaf area, number of primary branches, plant number of secondary branches by AMF compared to the control under drought stress. Similarly, Begum et al. (2019) reported that AMF-inoculated maize plants showed significant increase in height (36.32%) and dry weight (75.73%) over the control under drought stress. Drought stressed AM plants exhibited increased performance in terms of growth and biomass production, water and nutrient acquisition, and oxidative stress alleviation compared to control plants was reported by Essahibi et al., (2018).

In our study, combining biochar and AMF treatments improved plant height, shoot dry weight, root dry weight, the total root length, root volume, projected area, root surface area compared to the control and other treatments under drought stress condition. Our results were similar with a previous study which found that the combination of biochar and AMF increased plant growth (Hashem et al., 2019). Combination of biochar and AMF increased shoot and root biomass, leaf area meter, root surface area and root length in soursop (*Annona muricata* L.) seedlings (Harun et al. 2021). Similar results were reported by Budi and Setyaningsih (2013). They observed biochar and AMF significantly increased shoot dry weight, and root dry weight.

The present study demonstrated that drought stress reduced the nitrogen, carbon and chlorophyll content in the plants. Many previous studies found that drought stress decreased plant nutrients (Christophe et al., 2011; Rouphael et al., 2012; Heckathorn et al., 2014; Khalofah et al. 2021). Similarly, He et al. (2014) observed reduction in the concentration of nitrogen and phosphorus in plant tissue under drought stress. Total K and P uptake of maize organs showed a sharp decrease under drought stress (Ge et al., 2012). Drought reduced nitrogen and P content (Bista et al., 2018). Similarly, drought reduced chlorophyll content and leaf photosynthesis (Zhang et al., 2011; Hazrati et al., 2016; Bashri et al. 2021). This finding confirms earlier studies of Sawhney and Singh (2002) who observed that drought stress reduced chlorophyll *a*, *b* and total pigments in pumpkin. In drought stress reduced chlorophyll and carotenoid content in maize were demonstrated by Begum et al. (2019).

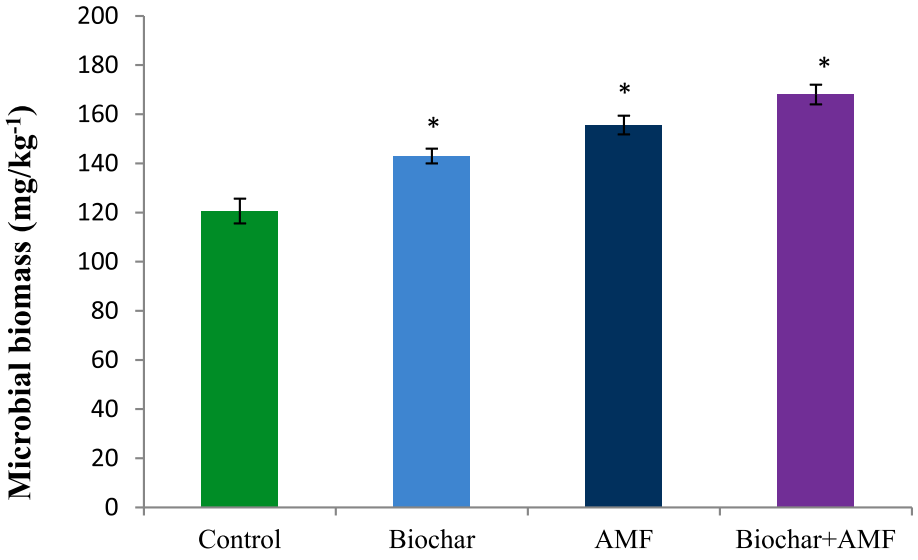

Fig. 5. Effect of drought stress on the microbial biomass in soil. Data are means of three replicates (n = 3), * asterisk differed significantly at P < 0.05*

Table 3
Effect of drought stress on soil enzymes activities.

Treatments	Alkaline phosphatase (μg $g^{-1}h^{-1}$)	Dehydrogenase activity (μg $g^{-1}h^{-1}$)	Fluorescein diacetate activity (μg $g^{-1}h^{-1}$)
Control	41.73 ± 0.64	32.00 ± 1.00	34.83 ± 0.76
Biochar	56.67 ± 1.15*	43.67 ± 2.21*	49.67 ± 1.53*
AMF	58.67 ± 1.53*	46.00 ± 1.00*	51.00 ± 1.00*
Biochar + AMF	65.00 ± 1.00*	54.00 ± 2.05**	59.00 ± 1.07**

Data are means of three replicates (n = 3), * asterisk differed significantly at P < 0.05*

In the present study, the biochar addition significantly increased the carbon content and the chlorophyll *a* content in okra compared to control under drought stress. Numerous researchers have reported that biochar application increased nutrient uptake (Kammann et al. 2011; Akhtar et al. 2014; Haider et al. 2015) and plant physiological properties (Haider et al. 2015; Lyu et al. 2016; Xiao et al. 2016) in various plants under water stress. Similar results were reported by Akhtar et al. (2014). Biochar significantly enhanced the photosynthetic rate, chlorophyll contents, stomatal conductance, relative water contents and water use efficiency in tomato leaves under drought stress condition. In another study, biochar (*Lantana camara*, 450 °C) increased the photosynthesis, the WUE, and Gs of okra (*Abelmoschus esculentus* L. Moench) under drought stress as compared to the control (Batool et al. 2015). Similarly, biochar improved photosynthesis and the water use efficiency of okra in drought stress.

The present study demonstrates that AMF treatment significantly enhanced the nitrogen and carbon content. Numerous researchers noticed that AMF inoculation improved plant nutrient and water uptake in various plants under drought stress (Gholamhoseini et al., 2013; Baum et al., 2015; Zhao et al., 2015a, 2015b; Bowles et al., 2018). A similar positive effect was reported with AMF treated maize showing enhanced nitrogen uptake under drought stress (Subramanian and Charest 1999). Wang et al. (2008) reported that AMF-inoculation increased uptake of minerals such as N, Mg and K in cucumber under water stress. Zhao et al. (2015a, 2015b) noted that AMF inoculation significantly increased P concentration in maize plants under drought condition. Similar results were obtained with AMF treated plants showing an increase in the plant physiological properties such as antioxidant enzyme activity and jasmonate synthesis of *Digitaria eriantha* under drought stress (Pedranzani et al., 2016). This finding confirms the observations of Abdel-Salam et al. (2018) who reported that AMF inoculation increased chlorophyll content and rate of photosynthesis in damask rose under drought stress condition. Gong et al. (2013) demonstrated that mycorrhizal seedlings had greater shoot dry weight, root dry weight, plant height, root length, instantaneous water use efficiency, net photosynthetic rate, stomatal conductance and photochemical quenching values when compared with non-mycorrhizal seedlings under water stress.

Combination of biochar and AMF resulted in significant enhancement in the nitrogen content, the carbon content and total chlorophyll content under drought stress. Similar findings were also noticed by Hashem et al. (2019), who showed that the combined application of AMF significantly increased total nitrogen content and total phosphorus content of shoot and root in chickpea under drought stress. Li and Cai (2021) reported that dual biochar and AMF increase the phosphorus content in maize under drought stress. Similarly, Hashem et al. (2019) has documented that the combined application of AMF and biochar significantly increased photosynthetic rate, relative water content chlorophyll *a*, chlorophyll *b* and total chlorophylls in chickpea under drought stress. Similar results have been reported by Li and Cai (2021) significantly enhanced the chlorophyll content and photosynthetic rate in maize under water stress.

This research demonstrated that drought stress decreased AMF spore number, microbial biomass and soil enzyme activities. Similar findings were reported by Geng et al. (2015) and Mariotte et al. (2015) drought strongly decreased soil microbial activity and fungal properties. Water stress reduced enzyme activities such as urease, protease activity, phosphatase activity and β-glycosidase activity in soil as reported by Sardans and Peñuelas (2005). Li and Sarah (2003) observed that drought stress decreased enzyme activities with increasing activity along a climatic transect in Israel.

The experiment demonstrated that biochar significantly improved the AMF spores number, the microbial biomass, the alkaline phosphatase, the dehydrogenase and fluorescein diacetate enzyme activities in soil under drought stress. Hashem et al. (2019) reported biochar treatment protected AMF from the deleterious effects of drought by improving the number of spores (36.73%), mycelium (79.68%), vesicles (28.65%) and arbuscules (28.55%) over drought stressed plants. Similar findings were also

noticed by Li and Cai (2021). Biochar application significantly increased microbial biomass by 38.0% and 65.9% under drought condition. Jabborova et al. (2020a) reported that biochar addition improved protease, acid phosphomonoesterase and alkaline phosphomonoesterase activities in soil. Similar result was confirmed by Ahmad et al. (2014). He found a stronger positive effect of biochar amendment on microbial biomass activity.

In a previous study, under drought stress AMF alone significantly increased the AMF spores number, the microbial biomass, the alkaline phosphomonoestrase, the dehydrogenase and fluorescein diacetate enzyme activities in soil. Similarly, Hashem et al. (2019) improved the number of spores of Arbuscular mycorrhizal fungi in chickpea under drought stress. Similar findings were also noticed by Li and Cai (2021) AMF inoculation improved soil microbial biomass. Qin et al. (2020) indicate that AMF can enhance the release of soil nutrients required for plant growth in response to increased soil enzyme activity.

However, combination of AMF and biochar strongly enhanced the AMF spores number, the microbial biomass, alkaline phosphatase, the dehydrogenase and fluorescein diacetate enzyme activities in soil compared to control and other treatments. Similar resulted were observed by Hashem et al. (2019) and Li and Cai (2021). Dual biochar and AMF inoculation significantly improved soil microbial activity, phosphatase activity by 40% in the maize rhizosphere under drought stress.

5. Conclusion

This study investigated the impact of biochar and AMF inoculation in mitigating the drought stress on okra. Under drought stress, biochar application could enhance root morphological traits viz: the total root length, the projected area, the root diameter and the root volume and soil enzyme activities. In drought stress, AMF could form a good symbiotic relationship with okra seedlings, and AMF symbiosis indeed improved root morphology, chlorophyll content, AMF spores number, microbial biomass, and improved the uptake of N. Combined inoculation with biochar and AMF clearly showed best results compared to the biochar alone and AMF alone treatments under drought condition. Dual application was more effective in enhancing plant growth, root morphological traits and chlorophyll content compared to other treatments. The AMF had synergetic effect with biochar for improving microbial biomass, AMF spores and enzymes activities in soil under drought stress. This finding reveals the prospective and potential use of okra combined with biochar and AMF for the successful crop cultivation under drought stress.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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