

Article

Interactive Impact of Biochar and Arbuscular Mycorrhizal on Root Morphology, Physiological Properties of Fenugreek (*Trigonella foenum-graecum* L.) and Soil Enzymatic Activities

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Abstract: Arbuscular mycorrhizal fungi (AMF) inoculation and biochar amendment has been reported to improve the growth of several crop plant; however, their role in stress amelioration individually as well as in combination has not been worked out. Limited information is available about the synergistic use of biochar and Arbuscular Mycorrhizal Fungi (AMF). Here, we investigated the synergistic effect of biochar and AMF on plant development, root architecture, the physiological performance of fenugreek (*Trigonella foenum-graecum*), and soil enzymatic activities. Biochar and AMF were shown to have a considerable effect on plant height, according to the data (53.3 and 66.6%, respectively), leaf number (22.5 and 45.1%), total root length (19.8 and 40.1%), root volume (32.1 and 71.4%), chlorophyll a content (26.0 and 17.8%), chlorophyll b content (50.0 and 28.9%), total chlorophyll content (30.0 and 18.1%), and carotenoid content (60.0 and 48.0%) over the control treatment. There was a considerable increase in plant height when biochar and AMF were combined together by 80.9%, total root length by 68.9%, projected area by 48.7%, root surface area by 34.4%, root volume by 78.5%, chlorophyll a content by 34.2%, chlorophyll b content by 68.4%, total chlorophyll content by 44.5%, and carotenoid content by 84.0% compared to the control. Our results recommend that the combination of biochar and AMF is advantageous in fenugreek growth, microbial biomass, and soil enzyme activities.

Keywords: plant height; nodule number; root length; chlorophyll content; relative water content; microbial biomass

1. Introduction

Medicinal plants are sometimes used in both traditional medicine and modern medicine [1–5]. Fenugreek (*Trigonella foenum-graecum* L.), a medicinal crop, forage legume,

and a traditional spice crop, belongs to the family Fabaceae [6]. The medicinal crop has been used traditionally in Indian Ayurvedic medicine, traditional Tibetan and Iranian medicine, Chinese medication, and modern medicine [7,8]. Seeds and leaves of fenugreek are useful in treating several diseases such as diabetes, cancer, head colds, influenza, constipation, bronchial complaints, asthma, emphysema, pneumonia, pleurisy, tuberculosis, sore throat, laryngitis, and fever [9–13]. Fenugreek seeds and leaves positively regulate blood sugar and blood cholesterol levels [14]. Seeds of fenugreek are a rich source of vitamins A, B1, and C, as well as carbohydrates (45–60%), proteins (20–30%), and fixed oils (5–10%) (mainly trigonelline (0.2–0.38%), choline (0.5%), saponins (0.6–1.7%), and volatile oils (0.015%). They are also a source of gentianine and carpaine, the flavonoids apigenin, luteolin, orientin, quercetin, vitexin, and isovitexin, the free amino acids arginine, histidine and lysine, and calcium and iron [15].

Recent trends in agricultural focus on minimising organic manure and expansion in usage of biofertilizer [16,17]. In addition to increasing soil health, organic manures provide the most important nutrients and micronutrients [18–20]. Biochar is eco-friendly and helps plant growth and development, as well as improving the physical, chemical, and biological properties of the soil and increasing the yield of various crops under different conditions [16–24]. Zhang et al. [25] reported that the composition of biochar includes carbon, nitrogen, hydrogen, and lower nutrient elements, such as K, Ca, Na, and Mg. It plays an important role in soil nutrient availability, adsorption, and soil enzyme activity [26–29]. He et al. [30] indicated that biochar enhanced the absorption of nitrogen (N), phosphorus (P), and potassium (K) by tomato. According to many studies, biochar treatment on its own has led to higher enzyme activity in soils with an acidic or alkaline pH, for instance, alkaline phosphatase, alkaline phosphomonoesterase, phosphohydrolase, and lipase-esterase [31–34]. AMF is known to improve the soil P mobilization and availability. Improved activities are an indication of the stimulatory effect of biochar and AMF on the residential microbial population, many of which will possess plant growth promoting (PGP) traits. Enzyme activity is the cumulative effect of long-term microbial activity and activity of the viable population at sampling. The overall dehydrogenase activity (DHA) of a soil depends on the activities of various dehydrogenases, a fundamental part of the enzyme system of all living microorganisms, such as enzymes of the respiratory metabolism, the citrate cycle, and N metabolism [35]. Numerous papers have revealed that biochar enhances overall plant development from germination to yield [36–39]. Saxena et al. [21] observed that the germination rate was higher when biochar was applied compared to the control. Rice straw biochar significantly increased plant height, the number of bolls per plant, average boll weight, and yield in cotton when compared to control treatment [40]. In biochar treatment, significantly higher Ca and Mg content in maize leaf samples was observed compared to untreated control samples [32]. Zhaoxiang et al. [41] found that the application of biochar has the effect of increasing root and shoot biomass of Ribwort. Biochar's impact on plant photosynthesis, chlorophyll content, and transpiration rate has been well-documented in several reports [29,34,42]. Sarma et al. [38] found that adding biochar with okra significantly improved the photosynthetic rate.

The integral importance of soil life in agricultural sustainability, including plant symbiotic associations, is steadily increasingly being recognized [16,17]. One of the prominent players among these symbioses is mycorrhiza, the common symbiotic combination of fungi and plant roots [43,44]. Arbuscular mycorrhizal fungi (AMF) play a major role in improving plant growth, plant nutrition, and soil enzyme activities while also promoting microbial activity [43–46]. Ascorbate peroxidase, catalase, and other enzymes are enhanced in plants inoculated with AMF [47]. Numerous researches have demonstrated that AMF treatment enhances root system branching, plant growth, and crop yield. [48–50]. Yaseen et al. [51] and Shokri and Maadi [52] stated that the rhizosphere microflora consists primarily of AMF, particularly prominent in natural environments. Thus, they play a crucial role in ecosystem function through biogeochemical cycles. Najafi et al. [53] observed that plant growth-promoting bacteria and AMF

co-inoculation might be useful for agriculture. Combined treatment with biochar and AMF has improved plant biometrics and soil enzyme activities. Using biochar with AMF has demonstrated the following: boosting plant growth, decreasing disease severity, and enhancing productivity. In maize, the application of biochar and AMF significantly improved phosphorus uptake [54]. Combined with 9% biochar, AMF significantly increased total root length and the number of very thin roots, fine roots, and thick roots in strawberry [55]. Plant biomass, leaf chlorophyll content and leaf nitrogen content in *P. australis* significantly increased by combined biochar and AMF treatment [56]. There is very little information about the interaction of biochar and AMF on fenugreek (*Trigonella foenum-graecum*). We aimed to test the following three hypotheses: (1) biochar and AMF can promote growth and root morphological traits of fenugreek; (2) biochar and AMF can improve physiological properties of fenugreek; (3) biochar and AMF can interact to enhance soil enzymatic activities and microbial biomass.

2. Materials and Methods

2.1. Materials (Seed, AMF, Biochar, and Soil)

The experiment was performed using field soil obtained from the Indian Agricultural Research Institute. The studied soil had the following agrochemical properties: pH—8.0, electrical conductivity 0.45 ds/m, soil organic carbon 0.41%, nitrogen 167 kg/ha, phosphorus 40.3 kg/ha, potassium 788 kg/ha. The biochar with a particle size >2 mm was purchased online (Amazon), and it was pyrolyzed at 400–500 °C from woody materials. From the Indian Agricultural Research Institute, the seed of fenugreek (Rajasthan Methi) and AMF were purchased.

2.2. Experimental Design

Fenugreek growth was examined using pot experiments in a green house at IARI's Division of Microbiology in New Delhi, India, to determine the effect of biochar and AMF. The entire experiment was conducted in a randomized block design with five repeats. In each replication, four plants have been taken for the mean). Biochar was applied at ~16 tons per hectare (1% w/v) to ensure the better growing of AMF in the soil. The experimental design was as follows; control (soil without biochar), biochar alone, AMF alone, and biochar + AMF. We used a pot experiment, mixing the soil with nothing, 1.0% biochar (or AMF). The experiment was carried out by introduction of AMF biofertilizer produced at IARI, Delhi, to the soil. The soil already may hold ineffective strains of native AMF. The AMF biofertilizer consists of 100 spores/g and 1200 IP/g. The AMF biofertilizer was layered at a depth of 5 cm from the surface of soil, ensuring 10 spores for each seed. Seeds were cultivated in plastic pots (20 cm diameter, 20 cm depth) containing 5.0 kg of soil. Each pot was watered once every 3 days. Soil temperature was maintained at 13–24 °C (day)/4–14 °C (night). The pot experiments in a net house started on 14 December 2020 and ended on 23 January 2021. Harvest occurred at 40 days, at which point morphological traits were all measured.

2.3. Measurement of Root Morphological Traits of Fenugreek

The root system was analyzed by a scanning system (Expression 4990, Epson, Los Alamitos, CA, USA). The digital images of the root were investigated by Win RHIZO software (Québec, QC, Canada).

2.4. Physiological Parameters Measurement

The relative water content (RWC) was measured in accordance with Barrs and Weatherly [57]. Photosynthetic pigments were measured according to the Hiscox and Israelstam method [58].

2.5. AMF Spores Soil Analysis

AMF spores were extracted from the soil by the wet sieving method. The spores were calculated by a microscope in accordance with Dare et al. [59].

2.6. Soil Microbial Biomass Analysis

Microbial biomass was measured in accordance with Vance et al. [60]. The absorbance of samples was measured at 280 nm.

2.7. Soil Enzymes Analysis

The alkaline phosphatase enzyme was analyzed according to Tabatabai and Bremner [61], while fluorescein diacetate hydrolytic was measured according to Green et al. [62]. Dehydrogenase enzyme activity was measured according to Casida et al. [63].

2.8. Statistical Analyses

One-way ANOVA was used to examine experimental data for multiple comparisons of HSD employing the Tukey test with StatView Software (SAS Institute, Cary, NC, USA, 1998). The significance of the effect of various treatments on plant growth parameters was determined by the magnitude of the p -value ($p < 0.05 < 0.001$).

3. Results

3.1. Plant Growth Parameters

The plant inoculated with AMF alone significantly improved the plant height by 53.3% in comparison to the control (Table 1), while the biochar alone significantly increased the plant height by 66.6%, the leaf number by 45.1%, and the nodule number by 35.9% as compared to control (Table 1). The combined application of biochar and AMF treatment significantly increased plant height by 80.9% over the control. The maximum leaf number was recorded and the combined treatment with biochar and AMF resulted in a 59.1% increase with respect to the control. Combined application of biochar and AMF treatment showed a positive effect on branch and nodule numbers, with a significant increase in the number of branches (35.8%) and nodule number (60.3%) over control (Table 1).

Table 1. Impact of biochar and AMF on plant growth parameters (plant height, leaf, branch and nodule numbers) of fenugreek.

Treatments	Plant Height (cm)	Leaf Number	Branches Number	Nodule Number
Control	15.00 ± 0.95 c	31.00 ± 1.00 d	4.66 ± 0.12 a	64.000 ± 2.80 c
Biochar	25.00 ± 0.91 a,b	45.00 ± 1.02 b,*	5.66 ± 0.15 a	87.00 ± 3.05 b
AMF	23.00 ± 0.58 b	38.00 ± 1.96 c	5.00 ± 0.12 a	82.33 ± 3.24 b
Biochar + AMF	27.14 ± 0.88 a	49.33 ± 2.03 a,**	6.33 ± 0.26 a	102.67 ± 4.40 a
HSD ≤ 0.05	2.44	2.28	1.84	5.01

Data are means of five replicates ($n = 5$), for each parameter, * asterisk differed significantly at $p < 0.05$ *, $p < 0.01$ **. The mean values ± SD followed by a different letter are significantly different according to Tukey's HSD range test at $p \leq 0.05$.

Data regarding the root fresh weight showed that AMF treatment significantly increased the root fresh weight by 54.2% compared with the control (Table 2). Treatment of biochar alone significantly increased the root fresh weight by 71.4% and the root dry weight by 25.0% compared with the control. However, the combined application of biochar and AMF showed a higher positive effect on root fresh weight (82.8%) and dry weight (37.5%) over control compared to a singular treatment with either component. There was also an improvement in shoot fresh weight (47.6%) and shoot dry weight (49.2%).

Table 2. Impact of biochar and AMF on fresh and dry weights of root and shoot of fenugreek.

Treatments	Root Fresh Weight (g)	Shoot Fresh Weight (g)	Root Dry Weight (g)	Shoot Dry Weight (g)
Control	0.35 ± 0.01 d	4.68 ± 0.09 d	0.08 ± 0.01 b	0.65 ± 0.01 d
Biochar	0.60 ± 0.01 b	5.36 ± 0.12 b	0.10 ± 0.01 ab	0.77 ± 0.01 b
AMF	0.54 ± 0.01 c	5.11 ± 0.14 c	0.09 ± 0.01 ab	0.72 ± 0.01 c
Biochar + AMF	0.64 ± 0.01 a	6.91 ± 0.20 a	0.11 ± 0.01 a	0.97 ± 0.01 a
HSD ≤ 0.05	0.0261	0.1408	0.0213	0.0483

Data are means of five replicates ($n = 5$), for each parameter, the mean values ± SD followed by a different letter are significantly different according to Tukey's HSD range test at $p \leq 0.05$.

3.2. Root Morphological Traits

Examination of root morphological traits revealed an increase in the total root length, root surface area, projected area, root diameter, and root volume in biochar treatment (Table 1). Biochar significantly increased the total root length 40.1%, projected area 22.4%, root surface area (23.6%), root volume (71.4%), and root diameter (20.3%) in comparison to the control, while plants inoculated with AMF gradually enhanced the total root length (19.8%) and root volume (32.1%). However, the combination of biochar with AMF increased the total root length (68.9%), projected area (48.7%), root surface area (34.4%), root volume (34.4%), and root diameter (31.0%) over control (Table 3).

Table 3. Impact of biochar and AMF on root morphological traits of fenugreek.

Treatments	Total Root Length (cm)	Projected Area (cm ²)	Root Surface Area (cm ²)	Root Volume (cm ³)	Root Diameter (mm)
Control	45.25 ± 2.01 d	5.80 ± 0.11 d	7.25 ± 0.20 d	0.28 ± 0.01 d	0.64 ± 0.01 d
Biochar	63.34 ± 2.70 b	7.10 ± 0.20 b	8.90 ± 0.38 b	0.48 ± 0.01 b	0.77 ± 0.01 b
AMF	54.16 ± 1.26 c	6.40 ± 0.15 c	8.10 ± 0.29 c	0.37 ± 0.01 c	0.71 ± 0.01 c
Biochar + AMF	76.37 ± 3.10 a	8.63 ± 0.29 a	9.68 ± 0.36 a	0.50 ± 0.01 a	0.84 ± 0.01 a
HSD ≤ 0.05	4.81	0.2387	0.3038	0.0213	0.025

Data are means of five replicates ($n = 5$), for each parameter, the mean values ± SD followed by a different letter are significantly different according to Tukey's HSD range test at $p \leq 0.05$.

3.3. Physiological Properties of Fenugreek

The effects of biochar and AMF application alone and in combination on the physiological properties of fenugreek are provided in Figures 1 and 2. Data in Figure 1 show that sole application of biochar significantly increased leaf contents of chlorophyll a by 17.8%, chlorophyll b by 28.9%, total chlorophyll by 18.1%, and carotenoid by 48.0%. As compared to the control, the AMF alone documented a 26.0% increase in chlorophyll a, 50% increase in chlorophyll b, 30% increase in total chlorophyll, and 60.0% increase in carotenoid content. The combination of biochar and AMF significantly enhanced the chlorophyll a, chlorophyll b, total chlorophyll content and carotenoid content by 34.2%, 68.4%, 44.5% and 84.0%, respectively (Figure 1).

3.4. AMF Spores Number, Microbial Biomass and Soil Enzymes Activity

Biochar and AMF treatments alone, as shown in Figure 2, increased leaf water content, although not significantly. The biochar-AMF treatment had the greatest leaf relative water content compared to the control.

The amount of AMF spores that were treated with biochar was 52.3 percent higher than in the control group (Figure 3). It was shown that AMF alone and in combination with biochar raised the spore count of AMF from 111% to 161%.

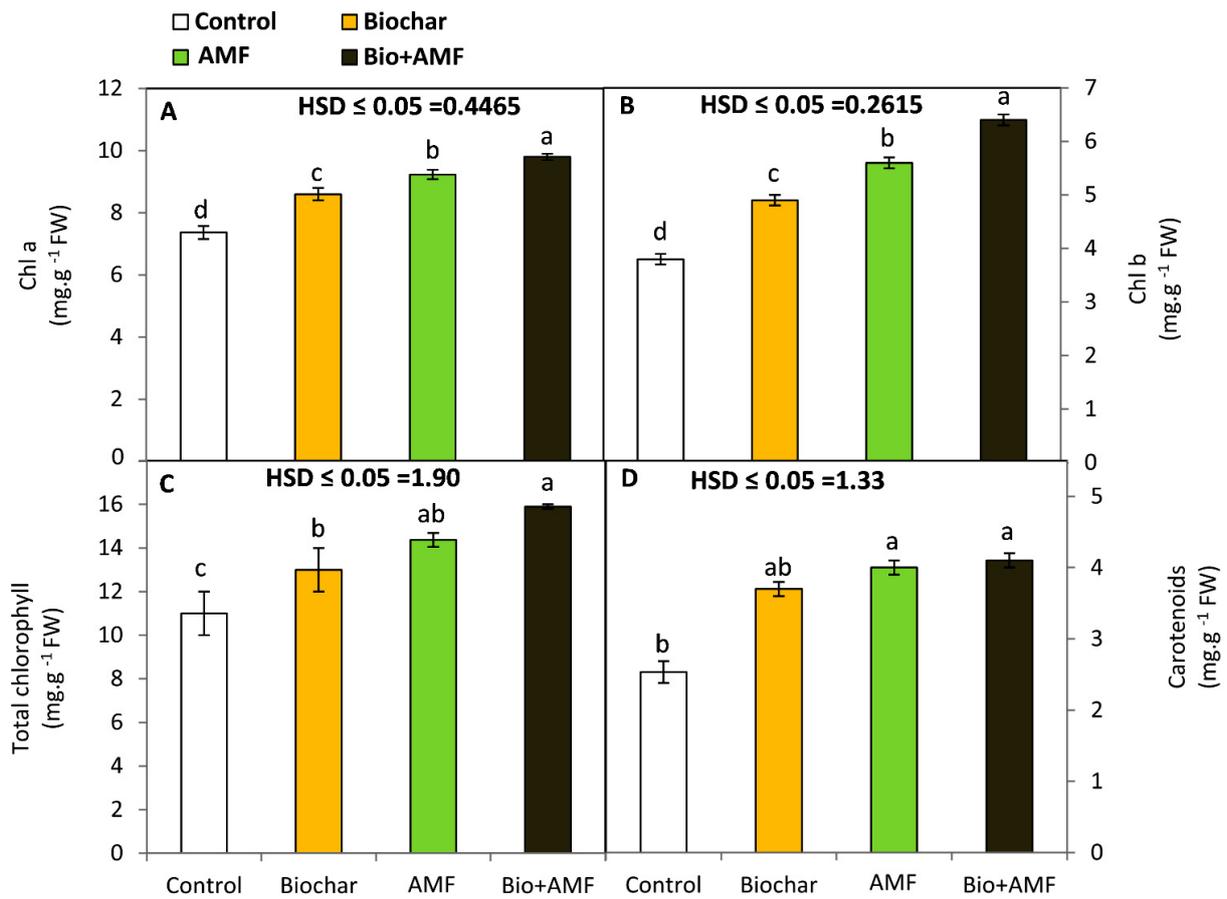


Figure 1. Impact of biochar and AMF on the photosynthetic pigments of fenugreek leaf. (A) Chlorophyll a, (B) Chlorophyll b, (C) Total Chlorophyll, (D) Carotenoid's content. Data are means of five replicates ($n = 5$), for each parameter, the mean values \pm SD followed by a different letter are significantly different according to Tukey's HSD range test at $p \leq 0.05$.

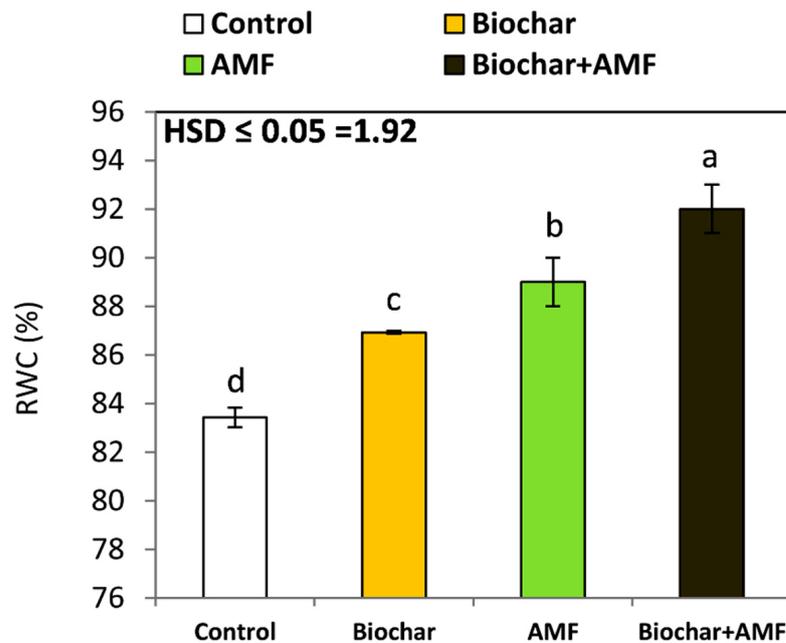


Figure 2. Impact of biochar and AMF on the relative water content of fenugreek leaf. Data are means of five replicates ($n = 5$), for each parameter, the mean values \pm SD followed by a different letter are significantly different according to Tukey's HSD range test at $p \leq 0.05$.

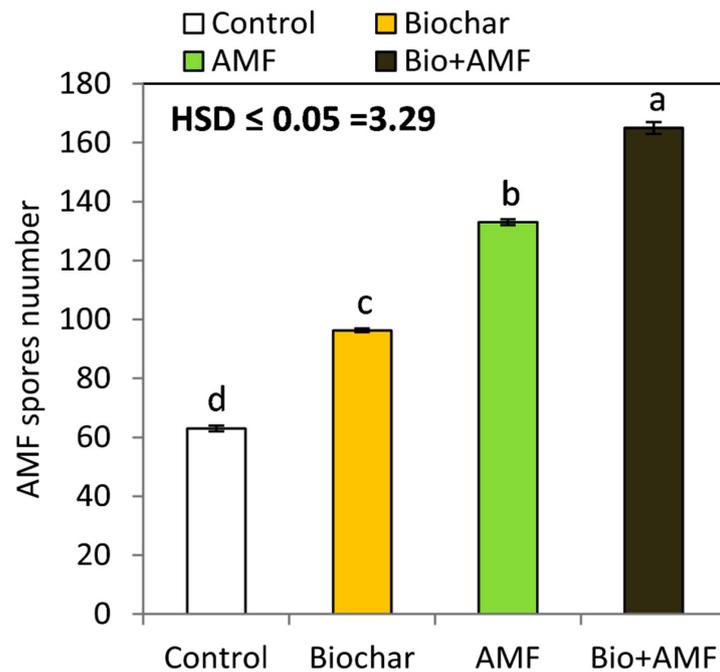


Figure 3. Impact of biochar and AMF on the AMF spores in soil. Data are means of five replicates ($n = 5$), and for each parameter, the mean values \pm SD followed by a different letter are significantly different according to Tukey's HSD range test at $p \leq 0.05$.

Figure 4 shows that biochar and AMF alone enhanced soil microbial biomass relative to the control. The microbial biomass increased by 44.4% and 54.6%, respectively, in biochar and AMF treatments over the control. The maximum increase over the control in microbial biomass (62.9%) was achieved in the treatment of biochar with AMF.

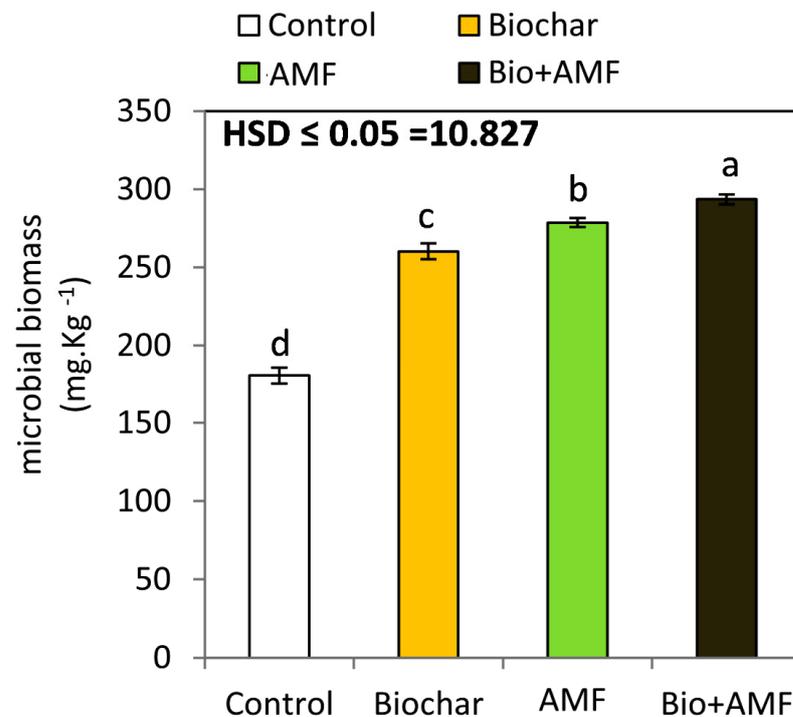


Figure 4. Impact of biochar and AMF on the microbial biomass in soil. Data are means of five replicates ($n = 5$), and for each parameter, the mean values \pm SD followed by a different letter are significantly different according to Tukey's HSD range test at $p \leq 0.05$.

In the present study, the alkaline phosphatase activity under either biochar or AMF treatments was enhanced by 25.4% and 43.6%, respectively, over the control (Table 4). The combined effect of biochar and AMF increased the alkaline phosphatase by 55.8%. The biochar and AMF treatments individually documented a significant increase in dehydrogenase activity by 21.5% and 41.7%, respectively, over control. However, dual application of biochar and AMF was more effective in enhancing the dehydrogenase activity (61.1%) compared to other treatments. Biochar alone gradually increased fluorescein diacetate activity in comparison with the control. The fluorescein diacetate activity increased by 34.6% (biochar) and 57.0% (AMF) treatments compared to the control. Treatment with biochar combined with AMF resulted in a greater increase in the activities of fluorescein diacetate activity (77.4%) compared to all treatments.

Table 4. Impact of biochar and AMF on soil enzymes activities.

Treatments	Alkaline Phosphatase ($\mu\text{g g}^{-1} \text{h}^{-1}$)	Dehydrogenase Activity ($\mu\text{g g}^{-1} \text{h}^{-1}$)	Fluorescein Diacetate Activity ($\mu\text{g g}^{-1} \text{h}^{-1}$)
Control	76.1 \pm 3.03 d	55.3 \pm 1.50 d	50.0 \pm 1.09 d
Biochar	95.5 \pm 4.10 c	67.2 \pm 2.42 c	67.3 \pm 2.65 c
AMF	109.3 \pm 4.21 b	78.4 \pm 3.06 b	78.5 \pm 3.13 b
Biochar + AMF	118.6 \pm 5.03 a	89.1 \pm 3.12 a	88.7 \pm 4.01 a
HSD \leq 0.05	3.34	4.79	5.15

Data are means of five replicates ($n = 5$), for each parameter, the mean values \pm SD followed by a different letter are significantly different according to Tukey's HSD range test at $p \leq 0.05$.

4. Discussion

4.1. Impact of Biochar and AMF on Fenugreek Plant Growth Parameters

Biochar application has revealed a wide range of benefits to plant growth through plant germination and development. In general, biochar treatment increased plant height by 66.6%, leaf number by 45.1%, and nodule number by 35.9% over control treatment (Table 1). Several researchers have reported that biochar application improves plant growth, development, and yield in different plants [29,30,32,34,39]. Similarly, Saxena et al. [27] found that root length, shoot length, and root biomass were positively impacted by biochar treatment, also seen by Zheng et al. [64], where biochar treatment increased the plant biomass of Chinese cabbage. This result is in line with that of the Gonzaga et al. study [65], who observed an increase in maize (*Zea mays* L.) plant growth when treated with coconut husk biochar compared to the control without any biochar. Qayyum et al. [40] observed that the rice straw biochar application significantly increased the plant height by 22.47%, the number of bolls per plant by 13.75%, average boll weight by 36.22%, and seed cotton yield by 14.48% compared with the control. Data regarding biochar alone treatment significantly increased the root fresh weight and the root dry weight by 71.4% and 25.0% over the control, respectively (Table 2). Similarly, Bopp et al. [66] reported that alkaline biochar significantly increased root growth, development, and root biomass. Trupiano et al. [34] found that morphological traits were enhanced in biochar applications (Figure 5). All reports discussed the beneficial effect that biochar is believed to help boost soil fertility by increasing soil acidity [27,30,32,34].

AMF are beneficial symbiotic fungi improving plant growth, development, and plant nutrient uptake in various crops. Data regarding the AMF treatment show a significant increase in plant height (53.3%), nodule number (28.5%), and root fresh weight (54.2%) in comparison to untreated plants (Tables 1 and 2). Several researchers report that AMF improved plant growth parameters of different plants [67–73]. Similar results were indicated by Fougnes et al. [74], where AMF increased plant growth, and nodulation of *Pterocarpus officinalis* Sharma and Kayang [75] reported that inoculating with AMF noticeably increased plant growth parameters such as number of leaves, leaf area, plant height, shoot length, root length, and root and shoot weight of tea (*Camellia sinensis* (L.)). They all refer to this

effect through improving water content and intercellular CO₂, P, and N contents [67–73]. The AMF can contribute to the increase in root dry matter in compacted soils, allowing decompression by improving the physical, chemical, and biological conditions of the soil, increasing the nutrient cycle and the efficiency of correctives and fertilizers [70–73].

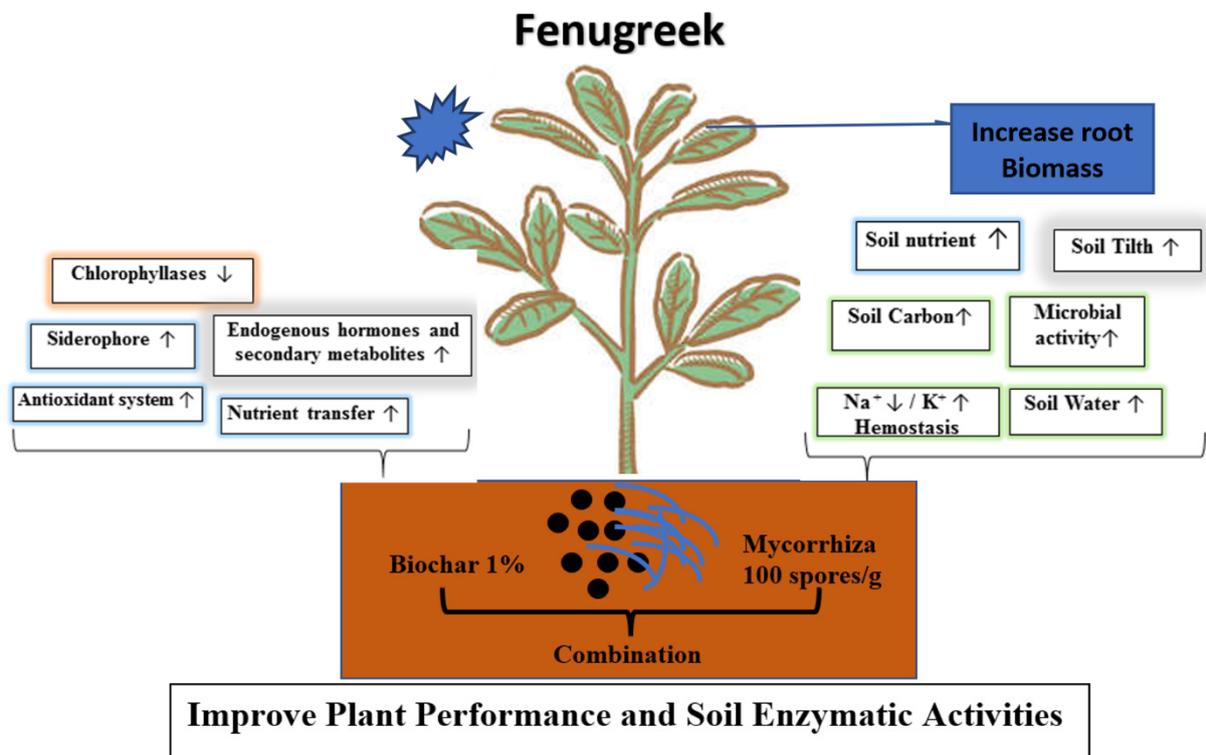


Figure 5. Summary of the mechanism of action for the joint impact of Biochar and AMF on plant development and soil enzymatic activity in the Fenugreek.

The application of biochar in conjunction with AMF treatment had a beneficial effect on plant height, leaf number, branches number, nodule number, and shoot/ root fresh and dry weight as compared to control (Tables 1 and 2). *G. etunicatum* and *G. margarita* were shown to have considerably increased height, diameter, shoot dry weight, and root dry weight relative to the control plants when both biochar and AMF were used [76]. The development of spinach, okra, and maize showed significance in the growth performance after the treatment with biochar and AMF [28,29,77]. The beneficial effect of the combined treatment starts from the biochar because the biochar trigger and enhance the AMF colonization. Therefore, the positive effect of the AMF is being doubled to three or four times due to the flourishing of the AMF and the development to the maximum limit under the best growth conditions. At the same time, the AMF synergistic effect with biochar by reaching the maximum soil conditioning to supply the best and optimum media for plant growth.

4.2. Impact of Biochar and AMF on Root Morphological Traits of Fenugreek

Root morphological characteristics were enhanced by biochar treatment compared to the control (Table 3). Numerous studies have shown that the addition of biochar to the soil improves plant root development [77–80]. Similar results were observed by Zheng et al. [64], where biochar application enhanced the root biomass and root system of *Brassica chinensis* L. (Chinese cabbage). Li and Cai [77] confirmed that biochar addition significantly improved root morphology. Trupiano et al. [34] also reported that the addition of biochar significantly improved plant root growth. This finding confirms earlier studies by Butnan et al. [81], who studied treatments of 1, 2, and 4% biochar application and their effect on improving maize dry weight and root length over the control treatment. At 1%, rice husk

biochar and woodchip biochar significantly enhanced the root volume and root length [77]. The addition of biochar improved the taproot length, the root volume, and the total root absorption area in tobacco [82,83] (Figure 5). It is not surprising that this result occurs because the biochar application increases plant growth and increases the demand for nutrients and water. Due to this, biochar is an excellent tool to fix nutrient deficiencies by absorbing nutrients, notably inorganic N [64,77].

AMF treatment increased root length, projected area, root diameter, and root volume in comparison to the control group. A similar positive effect was reported in tomato (*Lycopersicon esculatum*) seedlings, where AMF treatment enhanced the number of root tips and total root length [84]. The AMF inoculation enhanced root hair length and density. [85]. AMF inoculation had positive effects on the root growth of carrot and sorghum as reported by Kim et al. [86] when compared to uninoculated plants. It has been demonstrated by Budi and Setyaningsih. [76] that AMF treatment of the chinaberry significantly increased the diameter and length of both shoot and root. A study carried out by Singh et al. [68] indicates that inoculation of AMF enhances root growth, and AMF inoculation alone significantly changes the morphology of the roots [87]. Similarly, AMF significantly increased chickpea root length [88]. AMF can reduce root injury stress by altering root shape [89]. Padmavathi et al. [90] observed that AMF inoculation improved root length over the control.

As shown in Table 3, biochar and AMF significantly enhanced root system parameters, such as the total root length (68.9%), projected area (48.7%), root surface area (34.4%), root volume (78.5%) and root diameter (31.0%) as compared to the control treatment. Similar results were confirmed by Zhang et al. [83], where the combination of AMF and biochar, in addition to the root architecture, is also important in a plant's ability to utilize subterranean water and nutrients. This supports earlier findings by Hashem et al. [88], who reported that biochar in conjunction with AMF significantly enhances the length of chickpea root.

4.3. Impact of Biochar and AMF on Fenugreek Physiological Properties

The results presented in Figures 1 and 2 show that biochar treatment alone had a positive impact on fenugreek physiological parameters. Data in Figure 1 revealed that biochar treatment significantly increased chlorophyll a content by 17.8%, the chlorophyll b content by 28.9%, the total chlorophyll content by 18.1%, and carotenoid content by 48.0% over the control. Biochar application has been shown to boost plant photosynthesis, chlorophyll content, and transpiration rate in a variety of plants, and this study's findings are consistent with this earlier research [29,34,42,91]. Hashem et al. [88] found that biochar application enhanced the total photosynthetic pigments. Biochar application also significantly increased the photosynthetic rate by 27% and chlorophyll concentration by 16% compared to the control [92]. Similarly, Sarma et al. [38] found a strong positive effect of biochar amendment in the rate of photosynthesis in okra. Biochar is well recognized to significantly enhance the water and nutrient availability to plant roots, resulting in increased pigment synthetase and assimilation in plant leaves [29,34,42,91].

In Figure 5, the AMF treatment significantly improved the photosynthetic pigments. Our results agree with previous research by Hashem et al. [88], where total photosynthetic and carotenoid pigments improved in the presence of AMF, as well as earlier studies by Dell'Amico et al. [93] and Ren et al. [94], where AMF improved leaf area, stomatal conductance, and photosynthetic activity. In addition, AMF inoculation increases the chlorophyll content and the photosynthetic rate of maize by Li and Cai [77]. Padmavathi et al. [90] reported that AMF-inoculated *Rhizophagus irregularis* had improved chlorophyll content and reduced proline concentration in tomatoes (*Lycopersicon esculatum*) and bell peppers (*Capsicum annuum*). The researchers confirmed that AMF greatly increased antioxidant enzyme activity as well as net photosynthesis rate (Figure 5).

In the present investigation, the dual impact of biochar and AMF showed the greatest effect over all the other treatments on physiological parameters such as chlorophyll a content, chlorophyll b content, total chlorophyll content, carotenoid content, and relative water content (Figures 1 and 2). Dual inoculation significantly increased photosynthetic

pigments, and relative leaf water content (Figures 1 and 2). These findings confirmed with the research of Hashem et al. [88]. As shown in Figure 5, AMF and biochar promote siderophore formation, nitrogen fixation, and boost nutrient absorption and availability. Furthermore, they cause the production of endogenous phytohormones and antioxidants to be stimulated [89–93].

4.4. AMF Microbial Biomass, Spores Number and Soil Enzymes Activity

Supplementation of biochar helps improve the number of AMF spores, microbial biomass, and enzyme activities such as alkaline phosphatase, dehydrogenase, and fluorescein diacetate. Similar to previous work [26,88], based on the findings of this investigation, biochar improved the number of AMF spores by 52.3% (Figure 3), microbial biomass by 44.4% (Figure 4), alkaline phosphatase by 25.4%, dehydrogenase by 21.5%, and fluorescein diacetate activities by 34.6% in soil (Table 4). Biochar treatment has been demonstrated in several studies to increase AMF colonization rates [95–97], and several studies have shown that biochar significantly enhances esterase, lipase-esterase, trypsin, chymotrypsin, phosphohydrolase, and protease enzyme activities [33,34,98]. Similar work has shown that maize biochar improved soil enzyme activity [99], biochar application improved the activities of protease, acid, and alkaline phosphatase in soil [22], biochar application significantly increased urease activity by 40%, invertase activity by 9% and phosphatase activity by 46% at the highest biochar treatment rate (12 t ha⁻¹) [100], and that biochar application results in enzyme activity in soil. [39,101]. Gunal et al. [102] reported that 2% biochar treatment enhanced β -glucosidase activity in sandy loam soil. Biochar application increased microbial biomass carbon (MBC) content and phosphatase activity [103].

In the present study, AMF alone significantly enhanced soil numbers of AMF spores by 111.0% (Figure 3), microbial biomass by 54.6% (Figure 4), alkaline phosphatase by 43.6%, dehydrogenase by 41.7%, and fluorescein diacetate activities by 57.0% (Table 4). Similar results were observed by Li and Cai [77] and Ziheng et al. [104], where AMF inoculation improved soil microbial biomass and increased the enzyme activity in soil. These results are similar to previous work that showed that the combination of AMF and biochar treatment boosted soil microbial activity in the corn root and increased the amount of AMF spores in chickpea [77,88].

5. Conclusions

While treatment with only biochar or AMF alone improves fenugreek growth performance and physiological parameters. Combined biochar and AMF significantly enhanced microbial biomass, AMF spore numbers, and soil enzyme activities. This is the first investigation to report the effect of biochar and AMF on promoting fenugreek growth, root morphological traits, physiological parameters, and soil enzyme activities in non-fertile soils. These findings imply that a combination of biochar and AMF can enhance fenugreek growth, total chlorophyll content, carotenoid content, relative water content, soil microbial biomass, and enzyme activity. In the future, we plan to investigate the interactive effect of biochar and AMF on the plant growth and yield of fenugreek (*Trigonella foenum-graecum*) in field conditions.

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