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Biochar and Rhizobium Applications: A Promising Synergy for Improved Soybean Growth and Rhizosphere Microbial Activities

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Abstract

This study aimed to examine the effects of biochar application and rhizobium inoculation on soybean growth and specific rhizosphere soil microbial activities. Biochar, extensively tested over the past two decades, enhances soil physical, chemical, and biological properties, thereby positively contributing to agricultural yield. Leguminous plants like soybeans exhibit improved growth by reducing the use of mineral fertilizers through rhizobial inoculation. It is an important finding that the applications provided an approximately 100% increase in plant fresh weight. In this study, the utilized biochar served as an alternative organic material, not only regulating soil properties but also enhancing *rhizobial* activity. Conducted as a greenhouse experiment with the soils of Şanlıurfa, this research involved dual factor experiments with *rhizobium* applications and two doses of biochar (1%-1.5%) applications. According to the overall average results of the study, it was determined that *rhizobium* and biochar applications increased plant growth and nodule formation. While nodules did not form in samples without bacterial inoculation, this number increased to 13-19 with vaccination and applications.The determined values of CO_2 produce, DHA enzyme activity, and MBC content in the soil of the plant root zone increased with *rhizobium* inoculations and biochar applications. It was seen by correlation analysis that bacterial inoculation increased MBC contents in the soil, increased CO_2 content, and increased CO_2 increased total N, and was found to be significant.

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1. Introductıon

The need to feed a growing population inspired intensive agriculture practices and was motivated by commercial concerns over time. This has led to the attenuation of soil organic matter and microbial activities, hence falling below levels of soil fertility. In fact, studies point that enrichment in soil organic matter and restoration of the quality elements are promoted for sustainability; hence, even the recommendation tends to lie not only on mineral applications but on many incorporated microorganisms and organic material as well (Sarıoğlu et al., 2017). This research proves to be useful in zero waste projects and ecological studies since it gauges the extent of the contribution of biochar to rhizobial activity.

Pyrolysis is one of the thermochemical processes through which one leaves his plants to decay in very poor oxygen conditions and at very high temperatures (Zaman et al., 2017). During pyrolysis, energy sources like oil, coal, and natural gas form. Charcoal is also another form of energy (Akgül, 2017). Many researchers have identified the application of biochar obtained at pyrolysis temperatures between 300 and 1000 °C, resulting from pyrolysis on many organic materials under absence or at a very low percentage of oxygen, leading greatly to the improvement of physical, chemical, and biological properties of those soils (Utomo et al., 2016; Komkiene and Baltrenaite, 2016; Alaboz and Işıldar, 2018).

The use of biochar in agricultural uses has particularly increased in recent years, trying to mitigate some of the vicious effects of climatic alterations and to promote soil fertility sustainability by ecological restoration in agriculture (Wang et al., 2016).

On the other hand, biochar exhibits very high surface areas (about 500 m^2 g^{-1}) and is porous, hence the high cation exchange capacity and high-water retention ability (Lorenz and Lal, 2014). Additionally, biochar has a high oxygen content, which leads to high surface acid-base functional activities. Biochar helps in the provision of nutrients to the soil, making it possible for plants to take up certain elements easily from the soil, including phosphorus and nitrogen (Glaser et al., 2015).

A study was conducted to examine the influence of biochar from urban waste and sludge on soil properties from the developed soil material. According to the results, the content of microelements and heavy metals in the soil is affected by the biochar used (Fırat, 2023).

Although the exact full structure of biochar, produced from various organic material char, has not yet been fully elucidated, it was reported that it contains more concentric rings of fullerene type, or of short graphite structures, or semi-spherical structures than a single level long structure of graphite like normal coal (Ho et al., 2012). The application of high temperature to biomass enhances the breaking down of molecules to smaller, more aromatic forms. Biochar containing these forms may be persistent in the soils for a long duration compared to other organic materials. However, these activities in the soil have been attributed to biochar-soil application, but there is no clear understanding of their effect on the activities in the long run. Soils were taken from both areas: one area receiving a history of exposure to coal for over 150 years and normal agricultural fields. They were then incubated with time to know the changing microbial activities. The study showed that the agriculture practices and the characteristics of the different coals were significant in the activity of soil microbial. Thus, literature has it that any application of biochar without conducting joint soil and biochar property assessment may prove damaging to the soils.

Being a legume, proper inoculation of soybean with Rhizobium tends to provide better and high yields. The application of rhizobial inoculants in legumes also reduces the use of mineral fertilizers and chemicals, thus conserving the sustainability of agricultural soils (Naseer et al., 2019). Most of the rhizobial inoculums involve such organic material to bring out more bacterial activity, favoring root infection. The physicochemical properties of biochars are the best-suited for such rhizobial activities, so biochar can be used as a substitute for another scarce organic material. Experimentation involved various types of biochars preparations and inocula, with different numbers of days of storage. The storage period was found to influence the activity of the rhizobia. For instance, literature shows that solid biochar inoculum application resulted in a positive influence on plant growth (Glodowska et al., 2017). The experiment thus seeks to study the combined influences of biochar application and inoculation with rhizobium for soybean growth to benefit advancements of sustainable practices in the agricultural sector.

2. Materials and methods

This research was conducted in the greenhouse of the Department of Soil Science and Plant Nutrition at Harran University, using soil-filled pots with a capacity of 5 liters (5 kg soil). Some soil characteristics before the experiment are provided in Table 1.

Table 1. Some properties of experimental soil and biochar

Soil Properties	**Amount**		**Biochar Properties**	**Amount**
Texture	Clay-loam		pH	7.42
pH	7.61		EC, dS cm^{-1}	1.08
EC, dS m^{-1}	1.17		Total C%	56.02
Lime, %	31.52		Total N%	1.53
Organic matter %	2.05			
CEC (me $100g^{-1}$ soil)	38.5			
Phosphorus (mg kg^{-1})	15.92			
Fe, mg kg^{-1}	1.8			
Cu, mg kg^{-1}	0.6			
Zn, mg kg^{-1}	0.52			
Mn, mg kg^{-1}	1.3			

The study consisted of 2 *Rhizobium* variants (Non-R, R), 3 biochar variants (Non-BC, BC1, BC2), and 3 replications. The treatments were established as follows: control (no application), non- *Rhizobium* inoculation (Non-R), *Rhizobium* -inoculated (R), 1% biochar (BC1), and 1.5% biochar (BC2). The application pattern is given in Table 2.

Table 2. Application design

Application Design	
1. Application: Control	2. Application: R
3. Application: BC_1	4. Application: BC_2
5. Application: R+BC_1	6. Application: R+BC_2
Control: Non-application- R: *Rhizobium* inoculation- BC_1:1% biochar- BC_2:1.5% biochar	

The research applications were carried out together with soybean planting. The soybean seeds used in the experiment were GAPSOY 16, a registered soybean seed specifically developed for the region by the Southeastern Anatolia Project Agricultural Research Institute. Some properties of biochar derived from cotton stalks are provided in Table 1. Lime analysis in the research soils was conducted using the Scheibler calcimeter (Nelson, 1982). Cation exchange capacity (CEC) was determined through a method based on sodium acetate and ammonium acetate solutions (Sumner and Miller, 1996). Texture analysis was performed according to Bouyoucos (1951). Available phosphorus was analyzed following Olsen et al. (1954), and organic matter analysis was carried out based on Nelson (1982). Total nitrogen in soil was determined by Kjeldahl method (Bremner and

Mulvaney, 1982). Elemental analyses for Fe, Cu, Mn, and Zn were determined according to Chapman and Pratt (1982).

The seeds have been planted in each pot with a quantity of 5. Before the planting, 120 kg of (Güçdemir, 2006) DAP fertilizer per hectare (at a rate of 4.8 mg kg^{-1}) was applied, and no further fertilization has been conducted. On the 7th day after germination, the seedlings were thinned to 3 plants per pot. After the 32nd day, when the plants reached the flowering stage, analyses were performed. When the plants reached the flowering stage, three leaves with similar physiological development were selected, and chlorophyll content was measured using a SPAD meter to gather information. After the plant was gently removed from the pot, and the surrounding soil was cleaned. After obtaining the required soil sample, the roots were washed clean in a bucket of water. Subsequently, the plants were harvested, and root and above-ground samples were brought to the laboratory separately. For the determination of plant fresh and dry weights, the plants' fresh weights were recorded first. Then, the samples were kept in an oven at 70°C until a constant weight was reached to determine their dry weights.

2.1. SPAD values

The chlorophyll measurements of soybean plants were conducted using a SPAD meter. SPAD meter is a field-portable device to take chlorophyll content in the plant leaves. It is used to gauge the health condition and photosynthesis activity of the plant. The device measures the reflected green light from the leaf surface by a probe placed on the leaf and converts these data into the content of chlorophyll. The measurement was made at noon of the day.

2.2. Thermal camera images

The images used are high-resolution infrared pictures taken using a T650 camera (FLIR). The spatial resolution of the camera is 640 × 480 pixels, and the thermal sensitivity is rated at 0.04°C at 30°C.

2.3. Plant fresh and dry weights

The plants in each pot were separated from the root collar after harvest. Thereafter, the plants which were separated were partitioned into its components: root and above-ground section components, and fresh biomass of these parts was recorded. Drying of the plants is then done in an oven dried at a temperature of 70°C, where it is constantly monitored until it attains an equilibrium state. After the equilibrium state had been achieved, accurate weight records of the dried plants were taken.

2.4. Dehydrogenase (DHA) enzyme activity

The method of Thalman (1967) was used for DHA analysis. To the tubes sized 25 x 250 mm, 10 g of soil sample was taken. Then, 10 mL of TTC (Triphenyltetrazoliumchloride) solution was added to the sample, and the mixture was well stirred. The stoppers were put in the tubes, and they were kept in the dark for 24 hours. After 24 hours, the tubes' solution was filtered with the help of the filter paper, and the mixture was read with a spectrophotometer at the wavelength of 546 nm.

2.5. Microbial biomass carbon (MBC) content

The analysis of MBC content in the soil was conducted according to Öhlinger (1993). A 50g soil sample was adjusted to field capacity. The samples were placed in a desiccator containing 50 mL of chloroform. The desiccator was evacuated using a vacuum pump. After waiting for 24 hours, the samples were removed. The extracted samples were mixed with a potassium sulfate solution in a 1:5 ratio and left to shake for 30 minutes. After the shaking process, the samples were filtered, and the determination of organic matter was performed.

2.6. Soil respiration (CO_2 produce)

The analysis of CO_2 produce was conducted according to the method by Isermayer (1952). A 100g soil sample was placed in an Isermayer jar containing 50mL of barium solution, and the jar was sealed airtight. After 24 hours, a control sample without soil was also prepared. Phenolphthalein was added to create a red color. The red color was titrated with HCl until it turned colorless. The amount of acid used was noted, subtracted from the control, and calculated.

2.7. Catalase (CAT) enzyme ectivity

In the context of catalase enzyme activity, a mixture consisting of 10 mL of phosphate buffer with a pH of 7 and 5 mL of a 3% H_2O_2 substrate solution was introduced to a sample of 5 g soil. Subsequently, the amount of oxygen (O^2) released within a time span of 5 min, specifically at a temperature of 20 °C, was determined through the application of the methodology proposed by Beck in their 1971 publication.

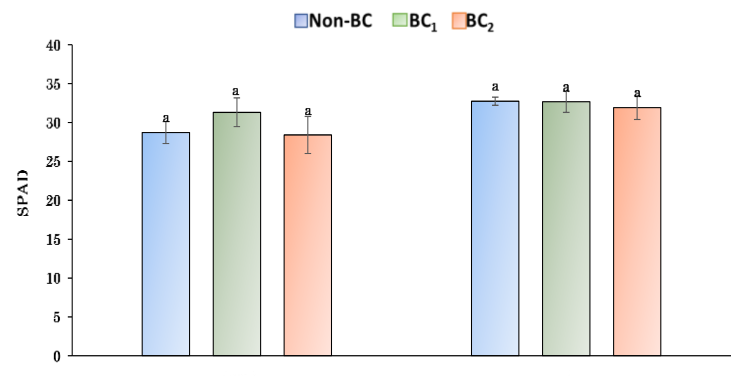
2.8. Analysis of data using statistical tools

The data obtained for each variable was subjected to the SPSS 21 software package to work-out ANOVA. To assess if significant differences exist between treatment means, the Duncan's Multiple Range test at $p<0.05$ was employed. Additionally, Pearson correlation coefficients were calculated to determine the direction and strength of relationships between the parameters. The selected values were illustrated with graphs at the end of the experiment (Köklü et al., 2006).

3. Results

3.1. Leaf SPAD value

In the study, leaf chlorophyll content was measured using a SPAD meter to gain insights into the effects of the applications. When examining the SPAD results (Figure 1), it can be observed that the applications increased plant chlorophyll content compared to the non-BC and non-R group. Although the biochar application led to an increase in chlorophyll content based on the averages, these results were found to be in terms of statistics insignificant ($p>0.05$). The applications involving *Rhizobium* inoculation raised leaf chlorophyll content from 28.70 to 32.73, indicating a 14% increase. Interestingly, the combined application of *Rhizobium* inoculation and biochar did not significantly alter the SPAD values. Leaf chlorophyll content serves as an important indicator of plant health.



	Non-R	R
Non-BC	28.70±1.40	32.73±0.51
BC₁	31.30±1.85	32.66±2.34
BC₂	28.40±2.38	31.90±1.51

Figure 1. SPAD values of soybean with *Rhizobium* inoculation (R) and biochar (BC) application (Mean ± S.E.); Different letters on bars exhibit significant difference between means within each variable. Non-R: without *Rhizobium* inoculation; R: *Rhizobium* inoculated; Non-BC: biochar not applied; BC1: 1% biochar applied; BC2: 1.5% biochar applied

3.2. Thermal camera images

At the end of the study, thermal images were captured from plants using an FLIR T650 thermal camera (Figure 2). It was observed that the plant temperatures varied between an average of 18-20°C, and these variations were determined to be statistically non-significant.

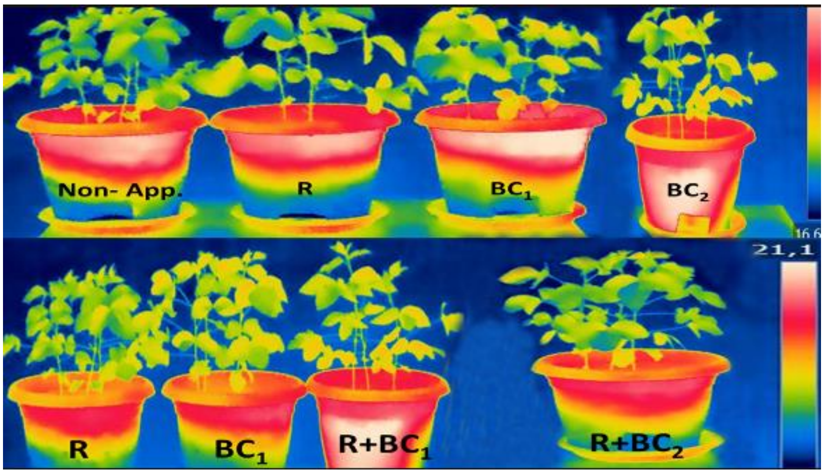
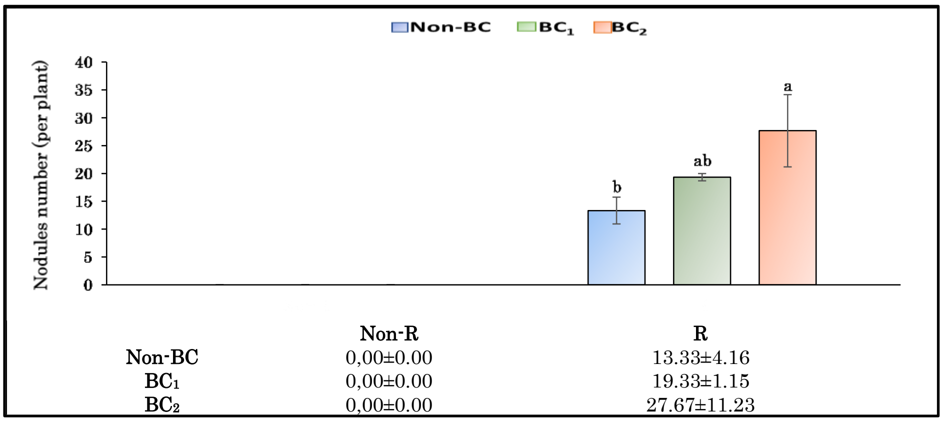


Figure 2. General thermal images of bacterial inoculation and biochar applications in soybean plants

3.3. Number of nodules

The results of nodulation, which is one of the significant indicators of nitrogen fixation, affected by the research applications are presented in Figure 3. According to the results, *Rhizobium* inoculation induced nodule formations, and in addition, biochar applications increased the number of nodules. Since nodulation did not occur in variants without *Rhizobium* inoculation, comparisons were made in *Rhizobium* and biochar variants. The BC1 application made in conjunction with *Rhizobium* inoculation increased the number of nodules by 45%, while the BC2 application resulted in a 107% increase, producing the highest nodulation. In the soil of Şanlıurfa, *Rhizobium* inoculation induced nodulation, and due to biochar applications, nodulation and consequently rhizobial nitrogen fixation were enhanced. These results obtained were found to be statistically significant ($p<0.05$).

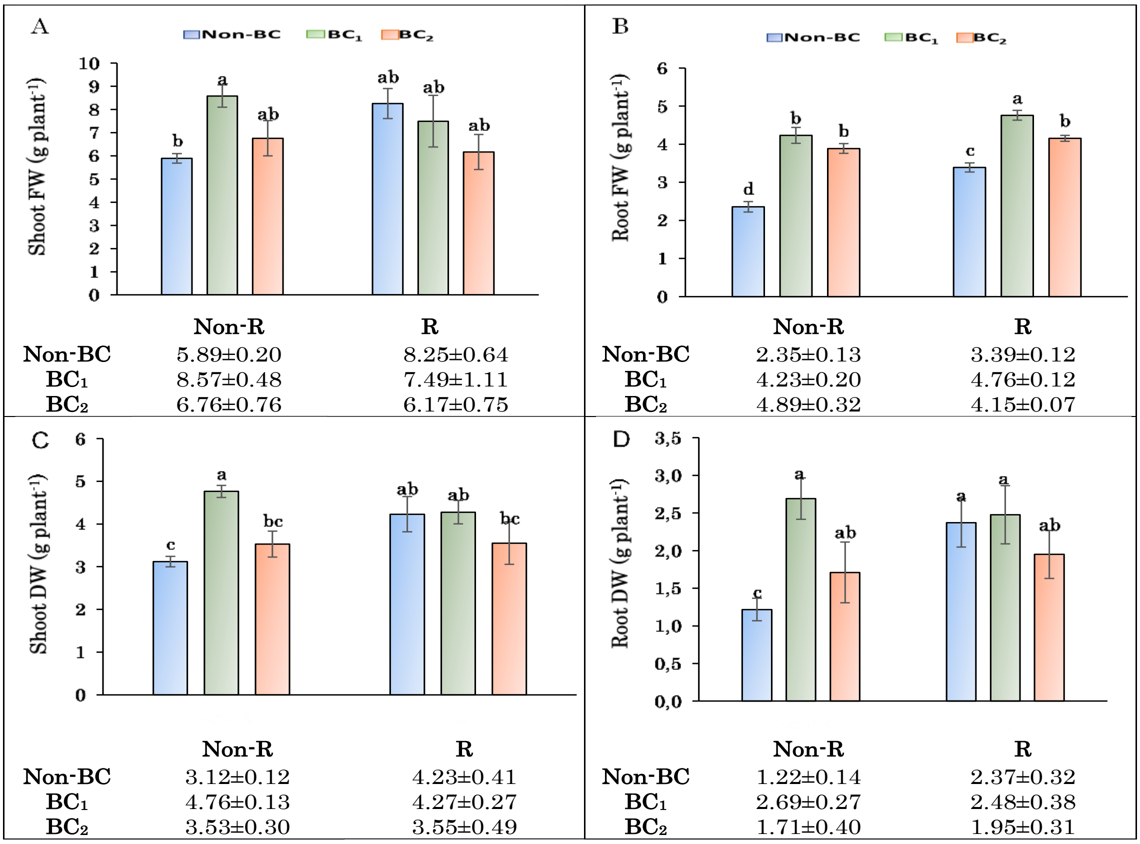


	Non-R	R
Non-BC	0,00±0.00	13.33±4.16
BC$_1$	0,00±0.00	19.33±1.15
BC$_2$	0,00±0.00	27.67±11.23

Figure 3. Number of nodules of soybean with *Rhizobium* inoculation (R) and biochar (BC) application (Mean ± S.E.); Different letters on bars exhibit significant difference between means within each variable. Non-R: without *Rhizobium* inoculation; R: *Rhizobium* inoculated; Non-BC: biochar not applied; BC1: 1% biochar applied; BC2: 1.5% biochar applied

3.4. Fresh and dry weight

Plant fresh weights are presented in Figure 4A-B. The treatments have significantly influenced the plant weights. As root and shoot fresh weights were considered, *Rhizobium* inoculation resulted in an average increase of 40%. Particularly, the BC1 application increased shoot fresh weight by 45% and root fresh weight by 79%. The combination of BC and *Rhizobium* in shoot fresh weight was lower compared to the individual application. However, in root fresh weight, the BC1+R application was determined as the highest value with a 101% increase. Overall, the treatments increased both root and shoot fresh weights when compared to the untreated control. These obtained values were found to be statistically significant ($p<0.05$).



	Non-R	R
Non-BC	5.89±0.20	8.25±0.64
BC_1	8.57±0.48	7.49±1.11
BC_2	6.76±0.76	6.17±0.75

	Non-R	R
Non-BC	2.35±0.13	3.39±0.12
BC_1	4.23±0.20	4.76±0.12
BC_2	4.89±0.32	4.15±0.07

	Non-R	R
Non-BC	3.12±0.12	4.23±0.41
BC_1	4.76±0.13	4.27±0.27
BC_2	3.53±0.30	3.55±0.49

	Non-R	R
Non-BC	1.22±0.14	2.37±0.32
BC_1	2.69±0.27	2.48±0.38
BC_2	1.71±0.40	1.95±0.31

Figure 4. Shoot fresh (A), Root fresh(B), Shoot dry (C) and Root dry (D) weight of soybean with *Rhizobium* inoculation (R) and biochar (BC) application (Mean ± S.E.); Different letters on bars exhibit significant difference between means within each variable. Non-R: without *Rhizobium* inoculation; R: *Rhizobium* inoculated; Non-BC: biochar not applied; BC1: 1% biochar applied; BC2: 1.5% biochar applied

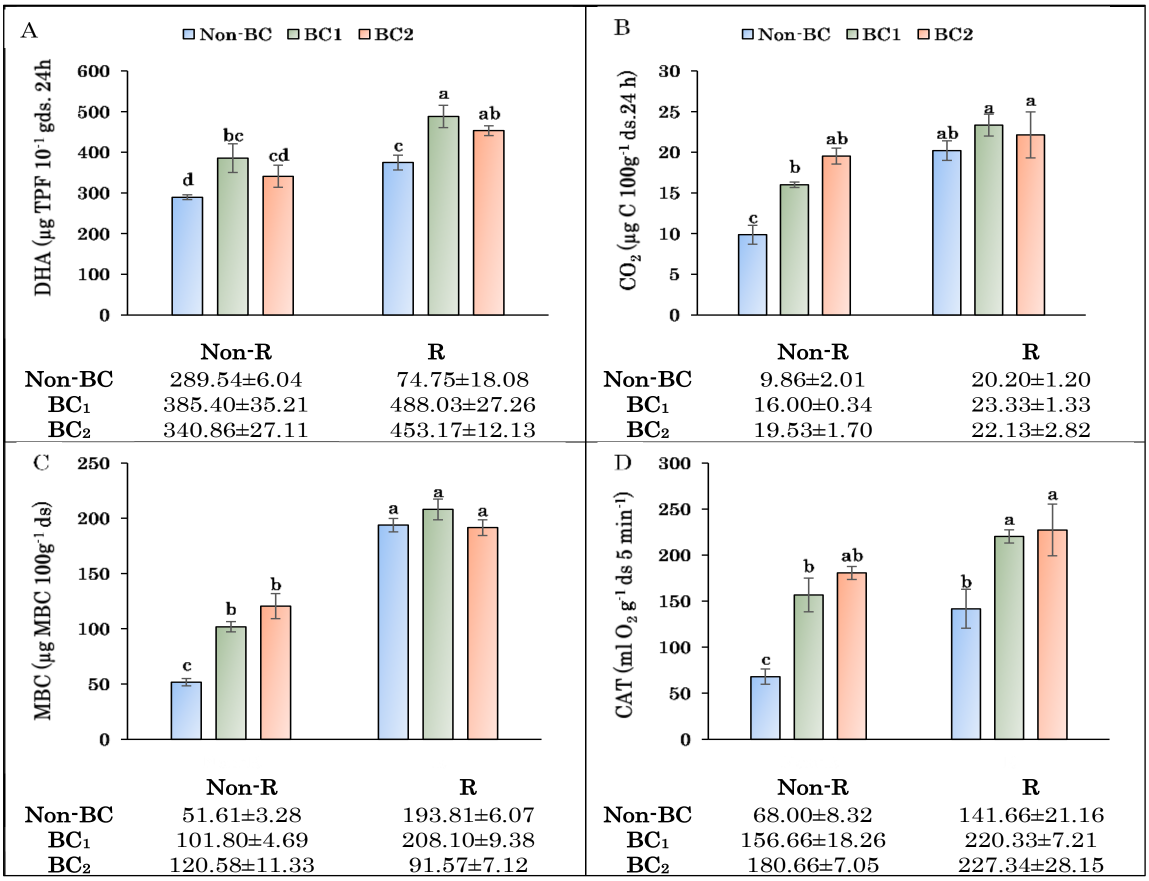
When plant root and shoot dry weights were examined (Figure 4C-D), in terms of shoot dry weight, the BC1 application without *Rhizobium* inoculation was found to be 52% higher compared to the untreated control, which was the best value observed. When *Rhizobium* inoculation was compared with the BC1 application, a 12% higher dry weight was determined. A similar situation is observed in root dry weight, indicating the significance of *Rhizobium* inoculation. The highest values found were, when compared to the untreated application, a 94% increase in the R application, a 121% increase in the BC1 application, and a 103% increase in the R+BC1 application, respectively. Overall, *Rhizobium* inoculation and BC applications led to an increase in root dry weights. The first dose of biochar applications resulted in higher root weight values compared to the second dose. These results obtained were found to be statistically significant ($p<0.05$).

3.5. Soil microbial activity

The effects of applications on dehydrogenase enzyme activity (DHA) are presented in Figure 5A. Similar effects to CO_2 results were observed for the applications.

Rhizobium inoculation increased DHA values. *Rhizobium* inoculation and BC applications supported this increase. The highest increase, with a rate of 68% compared to the non-treated group, was observed in the R+BC1 application. *Rhizobium* and biochar applications significantly increased DHA values in the root zone soil of soybean plants. These results were found to be statistically significant ($p<0.05$).

The effects of applications on soil respiration in the root zone of soybean plants, specifically the CO_2 production, are presented in Figure 5B. The lowest value was observed in pots without any application, while R, BC1, and BC2 applications increased CO_2 values by 105%, 62%, and 83%, respectively. BC applications alone increased CO_2 levels in the soil, and *Rhizobium* inoculation supported this increase. The highest value was recorded in the R+BC1 application. Furthermore, *Rhizobium* inoculation in the BC1 application led to a significant increase in CO_2 activity by 45% when compared to the BC1 application alone. These results were found to be statistically significant ($p<0.05$).



	Non-R	R
Non-BC	289.54±6.04	74.75±18.08
BC_1	385.40±35.21	488.03±27.26
BC_2	340.86±27.11	453.17±12.13

	Non-R	R
Non-BC	9.86±2.01	20.20±1.20
BC_1	16.00±0.34	23.33±1.33
BC_2	19.53±1.70	22.13±2.82

	Non-R	R
Non-BC	51.61±3.28	193.81±6.07
BC_1	101.80±4.69	208.10±9.38
BC_2	120.58±11.33	91.57±7.12

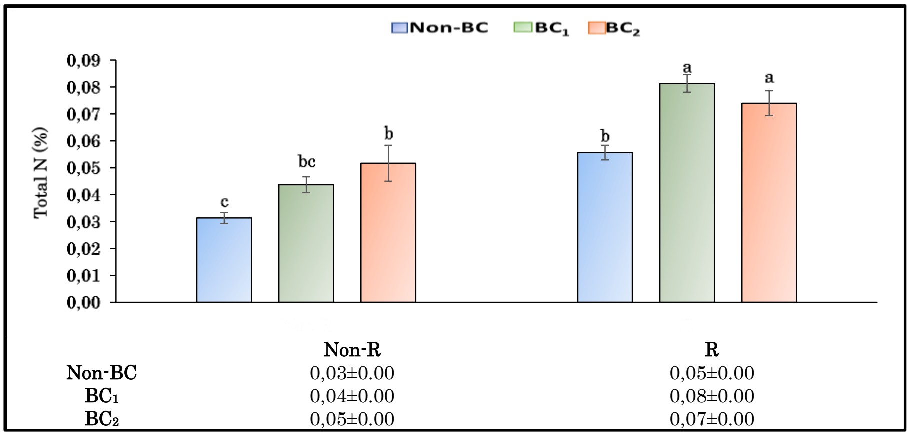
	Non-R	R
Non-BC	68.00±8.32	141.66±21.16
BC_1	156.66±18.26	220.33±7.21
BC_2	180.66±7.05	227.34±28.15

Figure 5. Dehydrogenase (DHA) enzyme activity (A), Soil respiration (CO_2) (B), Microbial biomass carbon (MBC) (C) and Catalase (CAT) enzyme activity (D) weight of soybean with Rhiz*obium* inoculation (R) and biochar (BC) application (Mean ± S.E.); Different letters on bars exhibit significant difference between means within each variable. Non-R: without *Rhizobium* inoculation; R: *Rhizobium* inoculated; Non-BC: biochar not applied; BC1: 1% biochar applied; BC2: 1.5% biochar applied

At the end of the study, the effects of applications on MBC content are presented in Figure 5C. *Rhizobium* inoculation increased the MBC content by 275% compared to the non-treated samples. BC applications supported this increase. The most significant increase, with a rate of 303%, was observed in the R+BC1 application. BC1 and BC2 applications also increased MBC values by 97% and 186%, respectively, compared to the non-treated samples. These results were found to be statistically significant ($p<0.05$).

Additionally, at the end of the experiment, the catalase enzyme (CAT), an indicator of microbial activity in soils, was investigated and is presented in Figure 5D. Upon examining the results, it was found that *Rhizobium* inoculation and BC applications individually increased CAT values by an average of 140% compared to the non-treated samples. In the combined applications of *Rhizobium* inoculation and BC, the highest results were obtained. These results were found to be statistically significant ($p<0.05$).

At the end of the trial, the results of the total nitrogen analysis are presented in Figure 6. *Rhizobium* inoculation and biochar (BC) applications have significantly increased the total nitrogen content. Compared to the parameters without application, R, BC1, and BC2 applications have resulted in increases of 77%, 39%, and 64% respectively. The most significant increase occurred with the R+BC1 application, reaching 159%. These results are statistically significant ($p<0.05$).



	Non-R	R
Non-BC	0,03±0.00	0,05±0.00
BC₁	0,04±0.00	0,08±0.00
BC₂	0,05±0.00	0,07±0.00

Figure 6. Total N of soils of soybean with *Rhizobium* inoculation (R) and biochar (BC) application (Mean ± S.E.); Different letters on bars exhibit significant difference between means within each variable. Non-R: without *Rhizobium* inoculation; R: *Rhizobium* inoculated; Non-BC: biochar not applied; BC1: 1% biochar applied; BC2: 1.5% biochar applied

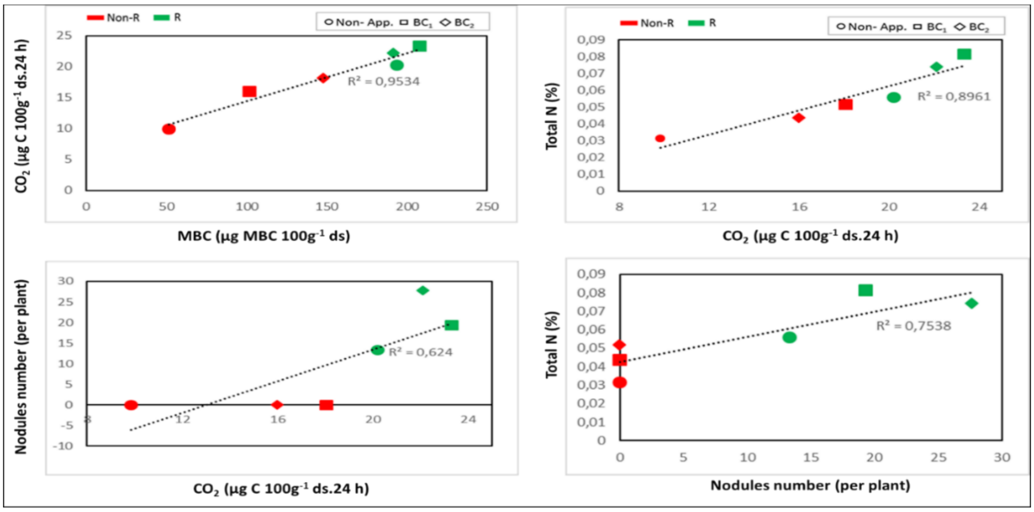


Figure 7. Correlation analysis relationship between the results obtained from the application. *Rhizobium* inoculation (R) and biochar application (BC)

At the end of the experiment, the measured parameters were subjected to correlation analysis to examine the relationships between them (Figure 7). The effectiveness of the R application is clearly visible in the obtained results. According to the results, there is a significant positive strong correlation between MBC and CO_2 (r= 0.826, p<0.05). There is also a significant positive strong correlation between CO_2 and Total N (r= 0.818, p<0.05). When nodulation efficiency was examined; there is a significant moderate-level relationship between CO_2 and the number of nodules (r= 0.695, p<0.05). The number of nodules had a strong significant positive correlation with Total N results (r = 0.816, p < 0.05). Results of the analysis indicated a positive relationship between MBC and CO_2, between CO_2 and Total N, and increased microbial activity due to a greater number of nodules with applications. On the other hand, there was a very strong positive correlation between the number of nodules and Total N results (r=0.816, p<0.05). From the above results, it can be summarized that the applications have a positive relationship between MBC and CO_2, CO_2 and Total N, and the number of nodules by increasing microbial activity.

4. Discussion

With these applications, great improvement was seen in plant growth and chlorophyll content. Leaf water content level is an important parameter to assure the healthy functioning of the photosynthetic activity and electron transport system (Wright et al., 2004). Both biochar and Rhizobium increase water usage efficiency of plants leading to more photosynthesis (Nadeem et al., 2017). Study evidence appears to support the fact that the application of biochar greatly augments the photosynthetic activity of plants based on their water use efficiencies (Akhtar et al., 2014; Baronti et al., 2014). Similar research results have found that Rhizobium together with biochar enhance the growth of soybeans (Tagoe et al., 2008). In fact, Rostami et al. (2008) observed in their study that the co-cultivation of corn with soybean, along with the application of biological fertilizer, increased the chlorophyll content. In another study conducted with soybean, it has been reported that chlorophyll content increased due to the application of Phosphobacteria, Rhizobium, and their combination treatments, which is in line with the present study (Selvakumar et al., 2012; Dixit, 2013).

In the study, thermal camera images were captured, and an investigation was conducted to determine if there were any differences among the applications. However, no significant differences were observed in the images. The use of thermal camera images is found in various studies. In a study by Tune (2016), the technique of thermal imaging was used to determine some physiological and morphological characteristics of cotton plants under different irrigation systems and nitrogen dosage conditions. In the results, except for the first measurement of factors such as blocks and irrigation water quantity, insignificant (p>0.01). It was emphasized that the use of thermal cameras did not have significant effects on nitrogen dosage. In another study, four different irrigation levels (S100, S75, S50, and S25) were applied to pepper plants, and physiological differences in the plants were attempted to be detected using thermal images. According to the findings, as the level of water stress in the plant increased, it was determined that the temperature also rose in the thermal images (Çamoğlu and Genç, 2013).

Bacterial inoculation, especially Rhizobium bacteria, is very important for nodule formation. Very importantly, in this study, the nodule formation was recorded in inoculated pots only. Some studies have indicated that inoculating the soybean plant with the nodule's specific bacterial strain contributes to effectiveness. Some studies done in the research, according to research that preceded the study, indicated that the formation had support from biochar application (Tilba et al., 2021). Recent research has confirmed that biochar can be applied as an alternative carrier material to peat for rhizobial inoculations. (Glodowska et al., 2017) Similarly, some studies (Ma et al., 2019) have shown that biochar applications improved nodulation and plant growth.

From this study, significantly different plant biomass weights were observed. The applied biochar shows an increasing trend in the obtained results, while the application of bacteria shows decreases in the results.

As per Yılmaz and Kurt (2018), combined applications of biochar and vermicompost really developed soil microbial activity. In general, however, other organic matter that is applied with biochar has proved to be much more useful for the soil and the plants. It may be due to the high C/N ratio of biochar for the reason for this situation. The application of biochar is important in the perspective of providing an environment to soil microorganisms rather than the direct scope of nutrients and energy provisions. Perhaps one of the reasons for biochars to give fluctuating results in plant biomass weights, when applied with Rhizobiums, may be associated with the high C/N ratio and the fact that it creates good conditions for other organisms in the soil.

This could be due to various reasons, for example, unequal distribution of biochar in the soil throughout, quality or quantity of biochar, etc., which can best be understood and explained after doing in-depth research in this respect. The biochar has a large surface area with high water-holding capacity, furthering the positive influence for plant development and growth. The same applies in the case of the microorganisms inoculated through micro-inoculation. Although there are studies on the combined use of microorganisms and biochar, they are limited (Ahmad et al., 2020). This was also supported by many research results, which indicated that biochar applications indeed increased nutrient uptake and plant growth (Egamberdieva et al., 2016; Ma et al., 2019; Dai et al., 2020). Biochar increases the uptake of vital nutrients, including N, P, and K, which are also contributors to plant development (Amin and Mihoub, 2021). Ducey and others (2013) add that biochar serves to bring about the improvement in supportiveness of life by microbial present within the soil, serving to bring about the healthiness of the soil for the growth of plants.

Soil application of biochar significantly affected microbial activity (Haddad and Lemanowicz, 2021; Wu et al., 2022). This study observed that application to soils results in an increase in MBC content in them (Silva et al., 2020). On the other hand, the application of biochar presents different impacts on soil microbial activities based on the type of soil, biochar, and application doses (Jin, 2010). Studies had indicated that, when applied with other organic and inorganic fertilizers, including soil conditioners, biochars readjust soil aeration to enhance better crop productivity, improve plant nutrients available to enhance the retention of nutrients, regulation of the pH of soil, increase in the Cation Exchange Capacity (CEC) of soil, and increasing microbial activity (Silva et al., 2020; Singh et al., 2023). In short, the help of MBC values indicated an increasing trend in microbial activity. In short, the current study brought similar results to the findings of so many such researchers. This kind of application of biochar directly or indirectly improves the value of soil microbial activities and microbial biomass. Further biochar applications of the soil contributed toward the positive development for soil microbial activity. In addition, the physio-chemical properties of the soil include physical properties of the soil characterized by porosity, bulk density, and surface area. The chemical properties include pH and nutrient elements contents (Li et al., 2018; Palansooriya et al., 2019).

Improvement in these cases can be furthered through the use of Rhizobium inoculation and biochar application to increase soil fertility, quality, or both at appropriate locations (Egamberdieva et al., 2018). This will be very useful, benefiting by a great extent to increase the physical, chemical, and biological properties of soils through biochar, as per studies (Zheng et al., 2019; Singh et al., 2022). In the other study, it was revealed that the use of biochar resulted in the biological improvement of the activity in the soil. It was determined that with biochar, Rhizobium applied individually or in combination with Rhizobium, a significant increase of nitrogen content in the soil occurred with all the indicators of biological activity. Biochar provides a large area for the storage of nitrogen due to its larger area (Li et al., 2018). High content of nitrogen by the fixing capacity of rhizobia, especially with legumes, makes the soil rich in available nitrogen (Kebede, 2021). This highly helps in improving the level of nitrogen in the soil.

5. Conclusions

For example, inoculation of Rhizobium bacteria in leguminous plants such as soybean proved to meet the nitrogen requirement of a plant and thus minimized mineral nitrogenous fertilizer application. However, the inoculation methods of Rhizobium may not work very well in each area for every kind of soil condition. Biochar produced in this study was used as a carrier material for organic matter and rhizobial inoculation. Rhizobium inoculation and biochar application, taken together, showed an improvement in plant biomass. No nodules were observed in soils where Rhizobium inoculation had not been carried out. Where the biochar applications were applied along with the inoculation of Rhizobium, the number of nodules increased. According to the results of the above-referred works, biochar was concluded as good soil conditioner, and it was a perfect carrier material for Rhizobium application.

Besides, the increased rhizosphere of the plant with Rhizobium inoculation and biochar application had improved parameters of CO_2 production, DHA activity, and MBC content. It showed that the mentioned practices have a positive effect on the biological nitrogen fixation soil microbial activity and growth of the plants.

The study asserted that biochar and Rhizobium have potential applications in sustainable agricultural strategies, whereby the methods improve soil fertility and support the growth of plants. More studies would need to be carried out to measure the impact of these applications under different climates and soil conditions, so that from its use, more concrete recommendations for augmenting agricultural productivity can be derived.

Declaration of Author Contributions

Dr. Ali SARIOĞLU have designed the study and collected the data. Dr. Ali SARIOĞLU executed the experiment. statistical analysis It was made by Emrah RAMAZANOĞLU. Dr. Ali SARIOĞLU wrote the article, and critically reviewed by Prof. Dr. Ahmet ALMACA and Prof. Dr. Kemal DOĞAN

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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