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Effects of biochar and bio-fertilizer on yield and qualitative properties of soybean and some chemical properties of soil

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Abstract

This study attempted to examine the effects of biochar amendment together with bio-fertilizer on soybean yield and its qualitative properties, as well as a few chemical properties of soil through a factorial randomized complete block design at three replications in east of Golestan Province (Iran) during 2014. The two factors under study included the following: (1) biochar amendment (in four levels of 0, 2.5, 8, and 16 tons per hectare), (2) bio-fertilizer containing phosphorus and sulfur growth-promoting rhizobacteria (in two levels of inoculation and non-inoculation) applied through foliar feeding. The results of analysis of variance indicated that interactions of biochar amendment and bio-fertilizer on harvest index and grain yield were significant ($p \leq 0.01$). According to the results of this study, the highest harvest index and oil content were 56.9, and 17.7%, respectively, in the treatment of 8 tons per hectare biochar and inoculation with bio-fertilizer. The lowest harvest index and the lowest oil content were in the control treatment. The interaction of biochar and bio-fertilizer on bulk density and cation exchange capacity was significant ($p \leq 0.01$). The results of this study demonstrated that biochar affected the amount of residual nitrogen in the soil after harvest, cation exchange capacity (CEC), acidity (pH), and electrical conductivity (EC). The highest grain yield (3440 kg/ha) was in the 8-ton biochar treatment with inoculated bio-fertilizer. Our study concludes that the biochar and bio-fertilizers can improve grain yield of soybean till 51% relative to the control.

Keywords Biochar · Bio-fertilizer · Soybean · Yield

Introduction

Soybean (*Glycine max* L.) is an annual, short-day plant from leguminous family. Soybean is a key crop worldwide, playing a critical role in public health owing to its oil and protein compounds (Chen et al. 2007). In recent decades, the escalated application of chemical fertilizers has given rise to serious environmental problems. Bio-fertilizers are considered an important source of nutrients in sustainable agriculture. Nowadays, it is crucial to apply various types of bio-fertilizers and soil amendments to maintain the balance of soil fertility (Mariya Dainya and Ushab 2016).

In recent years, biochar has been widely studied. Biochar as a stable, highly porous, fine-grained carbon compound is produced from pyrolysis of biomass under conditions of limited oxygen (Sohi et al. 2010; Abbas et al. 2018). With a greater effectiveness than manure, biochar can reduce the soil bulk density and enhance the water holding capacity in soils (Malik et al. 2018). This substance, with great potentiality in mitigating greenhouse gases, is capable of preserving carbon in soil for long periods (Lehmann and Rondon 2006). Additionally, biochar is produced and applied to achieve optimal management of agricultural waste. Moreover, biochar is vital because of its role in soil quality, including increased water holding capacity, cation exchange capacity, soil carbon content, enhanced aggregation, prevention of leaching, balancing the

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pH of acid soils, and preparing the conditions for further microorganism activity (Fowles 2007; Lehmann 2008; Downie et al. 2009; Laird et al. 2010; Ali et al. 2017).

Mukherjee et al. (2014) demonstrated the great impact of biochar on the cycling of elements and prevention of carbon, nitrogen, and phosphorus waste in the soil. They stated that biochar contained a diverse range of nutrients released at different rates and tremendously affected the soil fertility.

A few studies have reported that the application of biochar strengthened yield lower than control. In some cases, researchers have attributed such reduction to immobilization nitrogen by biochar (Rondon et al. 2007; Asai et al. 2009; Blackwell et al. 2009). This phenomenon is expected to persist for a relatively short time until the unstable component of biochar is degraded.

This study explored the effect of mixed application of biochar and bio-fertilizers on soybean yield and yield components and the quality of agricultural soils under study in an effort to fulfill sustainable agriculture.

Materials and methods

The field experiment was located at Yanqaq, which is a village in Galikesh, 10 km from Gorgan Province, Iran (37° 17′ N and 55° 21′ E).

The area is governed by a Steppe climate. The average annual temperature in Galicash is 17.3 °C. The average annual rainfall is 332 mm. This research was conducted as factorial under a completely randomized design with three replications. In fact, this study focused on several factors including biochar on four levels of 0, 2.5, 8, and 16 tons per hectare and bio-fertilizer in two inoculated and non-inoculated levels through one seed treatment stage and foliar feeding at the three- to four-leaf stage. The bio-fertilizer used was a set of different species of N-stabilizing bacteria, phosphate-solubilizing bacteria (*Bacillus coagulans*), soil pathogens controller, and plant growth-promoting rhizobacteria (PGPR) including *Azospirillum* spp., *Pseudomonas fluorescens*, and *Basillus subtilis*.

The physicochemical properties of soil in the field were specified on depths of 0 to 30 cm (Table 1). The samples were sieved through a 2-mm sieve and air-dried for 1 week. The designated soil physical and chemical properties were measured, using standard methods. Soil pH was determined by a 1:1 saturated paste method using a pH meter (McLean 1982). The soil electrical

conductivity (EC) was measured using a conductivity meter on an extract of soil obtained by shaking soil with deionized water at a 1:1 (*w/v*) soil/water ratio (Janzen 1993). The cation exchange capacity (CEC) was obtained by using the ammonium acetate method. The soil particle size distribution and bulk density of experimental soil samples were also obtained, using a hydrometer, and core methods, respectively (Gupta 2000). The organic matter was determined by the Walkley–Black method (Walkley and Black 1947; Nelson and Sommers 1982). The P, N, and K were measured by the procedures described by standard methods (Rowell 1994). Tillage and land preparation, including plowing, and double perpendicular disks and pre-planting leveling were completed. Then, the required fertilizers including organic sulfur (20 kg/ha), urea (200 kg/ha), and decaying organic fertilizer (10 t/ha) were added to the soil based on the pre-planting soil test. The zinc sulfate fertilizers (50 kg/ha) and manganese sulfate (30 kg/ha) were added to the soil simultaneously with planting.

The biochar was allocated and added to each plot by pushing aside 15 cm of topsoil. Each plot consisted of six 3-m rows spaced by 45-cm planting lines. The planting operation was manual at extraordinary density. After emergence at two- to four-leaf stage, the thinning operation was completed based on 8 cm plant distance. Before planting, the first stage of irrigation was completed to the field's full capacity. The subsequent irrigation was completed in four well-water stages based on soil moisture within 10-day intervals. During the growing season, the weed was removed manually in three states, sucking pest and soybean pod worms were chemically eliminated by Amide-chloride pesticides (250 cc/ha) and Fenpropathrin (1 l/ha).

The biochar used in this experiment was a mixture of poplar and plane tree wood prepared in the vicinity of the farm. At first, the wood was sliced into small chunks. Then, two large and small barrels were used to produce biochar. In fact, the small barrel was filled with small pieces of wood and then inserted into the large barrel upside down. Since a significant portion of the small barrel was filled with wood holding less oxygen, the outside surface of the smaller barrel (which is now inside the large barrel) was covered by wood and then ignited to generate heat. After half an hour of being exposed to indirect heat with limited oxygen, the pieces of wood inside the small barrel turned into biochar. Having been completely processed, biochar was sliced and then passed through 1 and 2 mm sieves. Finally, several chemical properties of biochar were specified (Table 2).

Table 1   A few physiochemical properties of the soil at testing site 0–30 cm deep

EC (dS/m)	pH	O.C (%)	Total nitrogen (%)	Phosphorus absorbable (ppm)	Potassium absorbable (ppm)	Clay (%)	Sand (%)	Silt (%)	Soil texture	B.D (g/cm^3)	CEC (cmolc/kg)
0.91	7.7	0.84	0.097	18.7	566	37	13	50	Silty loam	1.5	17.95

Table 2 Chemical properties of the biochar under study

EC (dS/m)	pH	Biochar total nitrogen (%)
2.85	9.6	0.38

The amount of residual nitrogen in soil was measured after harvesting soybeans from depths of 0–30 cm of each plot, while the soil nitrogen content was determined through Kjeldahl Nitrogen Analysis. The soil bulk density was determined by preparing intact samples of soils through cylinders. The soil acidity was determined through pH meter based on prepared saturation soil samples after harvesting soybeans. The cation exchange capacity was measured through replacement of sodium with all exchange cations found in the soil. Finally, the replaced sodium content was measured through flame photometer. The electrical conductivity (salinity) was measured firstly by saturation mud extracts from soil samples and then by EC-meter.

In order to measure morphological and functional traits at full maturity of soybeans, five plants per plot were randomly selected from the second, third, fourth, and fifth lines of each plot while removing half a meter from both ends of the planting lines. Then, all pods were counted to achieve the number of pods per plant, number of grains per pod, grain weight, and biological yield (after drying for 48 h at 70 °C). Finally, grain yield was measured in each treatment at harvest time through harvesting by sickle 1.5 m^2 of third and fourth lines of each plot including the borders (regardless of the plants half a meter top and bottom). Having been isolated from pod shells, the grains were separately placed in oven at 70 °C and moisture of 12% for 48 h. Afterwards, the grain yield was determined by a digital scale by distributing the grain dry weight (kg/ha). The harvest index was measured based on the ratio of grain yield to biological yield (%). The oil content of grain was extracted through Soxhlet with an industrial *n*-hexane solvent. The protein content was measured by Kjeldahl method. The

experiment was based on measurement of total nitrogen in the grains, assuming that nitrogen is entirely based on protein and the nitrogen-protein conversion ratio.

The data were analyzed by analysis of variance (ANOVA) with LSD test using the SAS program to determine the significance of differences between treatments. A *p* value less than 0.05 was considered significant and less than 0.01 as highly significant.

Results

The results of ANOVA on the traits demonstrated significant differences ($p \leq 0.01$) between biochar treatments in terms of residual nitrogen, bulk density (B.D), acidity (pH), EC, and CEC (Table 3). Furthermore, there were significant differences between the bio-fertilizer treatments in terms of bulk density, acidity (pH), and cation exchange capacity ($p \leq 0.01$). Concerning the interaction of bio-fertilizer in biochar, the ANOVA tables indicated significant differences in B.D, pH, and CEC ($p \leq 0.01$).

A comparative evaluation of mean values (Table 4) indicates that 16-ton biochar achieved the highest residual nitrogen in soil, pH, EC, and CEC. The highest soil bulk density was observed in the control treatment (biochar-free). The bio-fertilizer treatments indicated significant differences in terms of pH and B.D, whereas there were no significant effects on other traits.

The figures in each column with a common letter indicated no significant differences according to the LSD test. The results of ANOVA on yield and yield components of rapeseed (Table 5) indicated that the number of pods per plant, yield, harvest index, oil content, and protein content were significant among biochar treatments ($p \leq 0.01$). As for bio-fertilizer treatments, there was a significant difference only in the number of pods per plant ($p \leq 0.05$), while leaving no significant effects on other traits. Moreover, the interaction of bio-

Table 3 Analysis of variance for traits related to soil properties

		Mean square				
Sources of variations	DF	Residual nitrogen in soil	BD	Acidity (pH)	EC	CEC
Block	2	0.000002^{ns}	0.0000001^{ns}	0.0004^{ns}	0.00002^{ns}	0.026^{ns}
Biochar	3	0.000216**	0.0061458**	0.0619**	0.2020**	3.55**
Bio-fertilizer	1	0.000008^{ns}	0.0004593**	0.00453**	0.00001^{ns}	0.821**
Interaction of bio-fertilizer in biochar	3	0.000002$^{n.s}$	0.000088**	0.01644**	0.00049*	0.419**
Test error	14	0.000001	0.000003	0.0017	0.00011	0.0103
Coefficient of variation	–	1.3	0.11	0.16	0.86	0.55

n.s, *, and ** represent insignificant difference, and significant difference at probability levels of 5 and 1%, respectively

Table 4 Comparison of mean values for traits related to soil properties

Treatment		Residual nitrogen in soil (%)	B.D (g/cm^3)	Acidity (pH)	EC (dS/m)	CEC (meq/100 g)
Biochar (t/ha)	Biochar-free	0.092 [c]	1.59 [a]	7.66 [d]	1.03 [d]	17.36 [d]
	2.5	0.096 [b]	1.54 [b]	7.75 [c]	1.12 [c]	18.15 [c]
	8	0.103 [a]	1.53 [c]	7.85 [b]	1.22 [b]	18.46 [b]
	16	0.105 [a]	1.51 [d]	7.89 [a]	1.45 [a]	19.22 [a]
Bio-fertilizer	Non-inoculation of bio-fertilizer	0.098 [a]	1.55 [a]	7.80 [a]	1.21 [a]	18.48 [a]
	Inoculation of bio-fertilizer	0.099 [a]	1.54 [b]	7.77 [b]	1.21 [a]	18.11 [a]

Mean values with the same letters in each column indicated no satistically significant differences

fertilizer in biochar indicated significant differences in the number of pods per plant, yield, and harvest index ($p \leq 0.01$).

Discussion

Characteristics that were measured on the soil include the remaining nitrogen, bulk density, cation exchange capacity, and electrical conductivity, which are discussed in detail.

Residual nitrogen in soil

The results revealed that an increase in the amount of biochar increased the residual nitrogen in soil. Subsequently, the nitrate leaching was mitigated in the growing season by increasing the amount of biochar. The greatest amount of residual nitrogen (Fig. 1) was 0.11% achieved by the 16-ton biochar, indicating a 14% improvement compared to the biochar-free treatment (0.09%). Fowles stated that biochar can prevent the leaching of nutrients, especially nitrates (2007). Biederman and Harpole similarly found that application of biochar minimized the leaching of soil nutrients and enhanced the plant yield, while advising to continue studies in this regard (2012).

The large specific surface, internal space, and the polar and non-polar surfaces have led to high capability of biochar in absorbing organic molecules and different elements. Since biochar stimulates microbial activity, particularly mycorrhizal fungi, biochar can remarkably affect the cycling of nutrients (Ishii and Kadoya 1994; Steiner et al. 2008; Lambers et al. 2008). In a study on the effects of biochar and two species of mycorrhizal fungi on nitrate leaching under corn cultivation, it was found that biochar prepared from rice bran reduces nitrate leaching by up to 63% compared to the control (Kamara et al. 2015).

Soil bulk density

The comparison of mean values indicated that the highest soil bulk density was 1.56 g/cm^3 achieved by biochar-free non-inoculated treatment, whereas the lowest bulk density was 1.51 g/cm^3 in the 16-ton biochar inoculated with bio-fertilizer (Fig. 2).

In terms of physical properties, biochar is quite porous weighing much less than farming soil. For that reason, the addition of biochar to soil affects its pore network, enhancing the total surface area of soil (Chen et al. 2007).

Given its stability against degradation, biochar seems to be able to directly enhance the water holding capacity in the long term owing to multiple pores and reduction of soil bulk density (Lehmann and Joseph 2009). In a research, it was reported

Table 5 ANOVA on effects of nitrogen fertilizer and cultivar on yield and yield components of soybean

Mean of squares								Df	Sources of variations
Protein content	Oil content	Harvest index	Biomass yield	Grain yield	1000 grain weight	Number of grains per pod	Number of pods per plant		
0.34[ns]	0.29[ns]	0.000002[ns]	3490339[**]	961[ns]	0.66[ns]	0.00041[ns]	8[n.s]	2	Replication
13.54[**]	5.04[**]	0.00291[**]	486724[ns]	1568659[**]	0.27[ns]	0.00263[ns]	117[**]	3	Biochar
5.92[ns]	1.04[ns]	0.00001[ns]	159[ns]	3384[ns]	0.07[ns]	0.00375[ns]	6[*]	1	Bio-fertilizer
1.00[ns]	0.15[ns]	0.00021[**]	8198[ns]	7059[**]	0.08[ns]	0.00041[ns]	7[**]	3	Biochar × bio-fertilizer
1.86	0.48	0.00001	209,610	891	0.31	0.00184	1.14	14	Test error
3.5	2.4	0.59	7.96	0.96	0.43	2.1	2.4		C.V (%)

ns, *, and ** represent insignificant difference, and significant difference at 5 and 1%, respectively
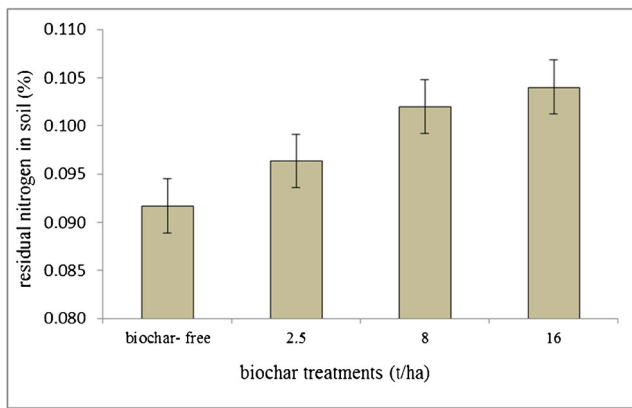
Fig. 1 Effect of biochar on residual nitrogen in soil. Each value is the average of 3 replicates. Vertical bars indicate +/- standard error. Different letters indicate a significant difference (P<0.05)

Fig. 3 The interaction of bio-fertilizer in biochar on the soil acidity. Each value is the average of 3 replicates. Vertical bars indicate +/- standard error. Different letters indicate a significant difference (P<0.05)

that biochar was more effective than manure in reducing soil bulk density and enhancing the water holding capacity (Laird et al. 2010).

In a study on the effect of mycorrhiza, sewage sludge, and the output biochar on the physical structure and quality of soil under maize cultivation in Shahin Shahr, (Isfahan), Ebrahimi found that the combined use of mycorrhizal fungi and biochar improves pore size distribution and physical quality of the soil while enhancing the stability of soil structure and reducing erosion (Ebrahimi et al. 2012).

Soil acidity (pH)

One of the most important chemical properties of soil is acidity. The results indicated that the combined effects of biochar and bio-fertilizers on acidity of soil under soybean cultivation were significant ($p \leq 0.01$). A comparison of mean values for treatments (Fig. 3) reveals that the lowest acidity is 7.57 achieved by the biochar-free treatment with inoculated bio-fertilizer while the highest
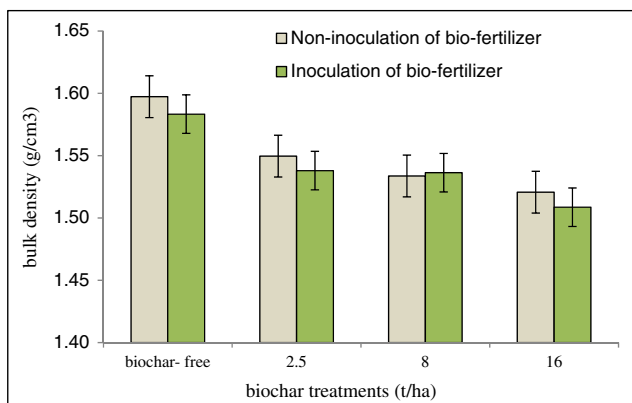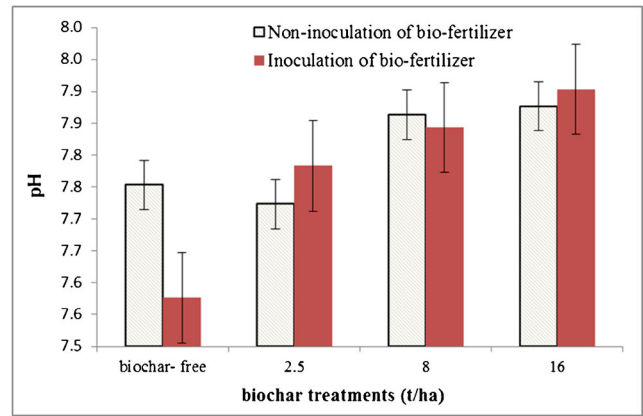
acidity is 9.70 in the 16-ton biochar treatment with inoculated bio-fertilizer. The increased acidity of soil was quite expected by addition of biochar with regard to acidity of biochar (9.62) prior to addition to the soil (Table 2) and acidity of the farming soil prior to harvesting (7.70). Biochar can facilitate the higher activity of microorganisms in acidic soils by raising pH (Thies and Rillig 2009).

Field studies conducted in the long run have obtained evidence that the chemical state of biochar transforms over time when it has been added to the soil. Fresh biochar mainly consists of a crystalline phase and an amorphous phase with aromatic structures (Lehmann et al. 2005). After mixing biochar with the soil, the outer surfaces of particles experience oxidation and react with different soil structures. The outer surfaces contain different functional groups (including O-H) exposed at maximum oxidation (Lehmann 2007).

In a study on the effects of three types of biochar on a few chemical and physical properties of soil, there was a significant increase ($p < 0.01$) in soil acidity (Najmi et al. 2014).
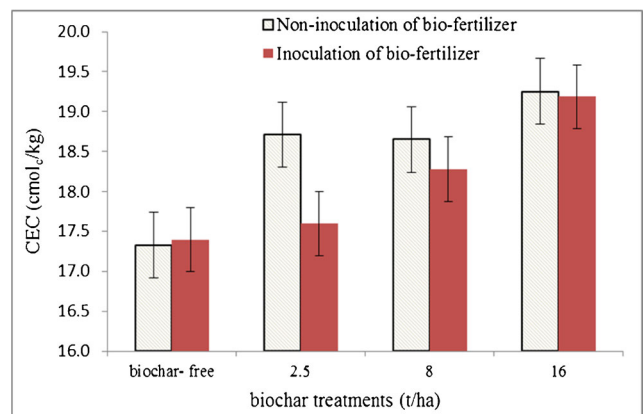

Fig. 2 The interaction of bio-fertilizer in biochar on the soil bulk density. Each value is the average of 3 replicates. Vertical bars indicate +/- standard error. Different letters indicate a significant difference (P<0.05)

Fig. 4 The interaction of bio-fertilizer in biochar on CEC. Each value is the average of 3 replicates. Vertical bars indicate +/- standard error. Different letters indicate a significant difference (P<0.05)
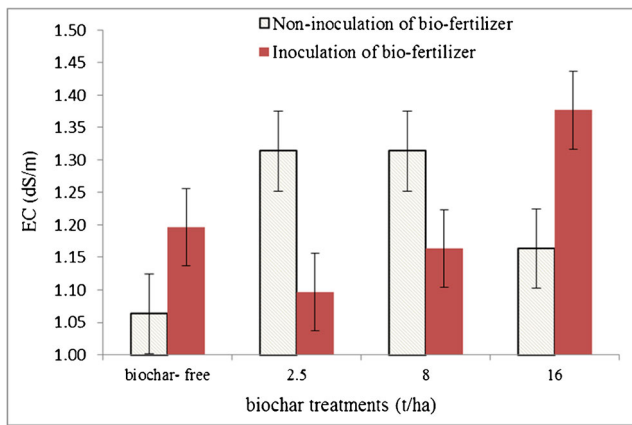
Fig. 5 The interaction of bio-fertilizer in biochar on EC. Each value is the average of 3 replicates. Vertical bars indicate +/- standard error. Different letters indicate a significant difference (P<0.05)

Cation exchange capacity

Comparison of mean values for different treatments showed that the highest CEC of soil under soybean cultivation was 19.25 meq/100 g in the 16-ton biochar treatment with non-inoculated bio-fertilizer, whereas the lowest was 17.32 meq/100 g in the biochar-free treatment with non-inoculated bio-fertilizer (Fig. 4).

CEC is one of the important soil properties used as an input variable in environmental models (Keller et al. 2001). The range of CEC variations is less than 1 cmolc/kg for sandy soils with low organic matter and over 25 cmolc/kg for clay soils with high organic matter (Mirkhani et al. 2005).

Studies on tropical soils across South America (Brazil), where soils essentially contain minimal CEC, suggested that addition of biochar increased CEC. This in turn mitigated the leaching of soil nutrients (Glaser et al. 2001). Biochar is not effective only in the CEC of highly weathered soils. Adding 2% biochar to Malisols soil (steppe grasslands and lawns) with low cation exchange capacity reported about 17% increase in soil CEC after 500 days (Laird et al. 2010).

Biochar, compost, and their mixture amendments significantly improved soil water content, OC, N, P, and CEC (Agegnehu et al. 2016).

Electrical conductivity (EC$_e$)

Electrical conductivity (salinity) is one of the key characteristics of water often measured for chemical analysis of water. Similarly, studies on soil salinity measure the electrical conductivity of soil saturation extract (EC$_e$).

The comparison of average interaction of bio-fertilizers and biochar treatments on EC suggests that biochar treatments statistically fall on four different levels, where an increase in biochar intensifies the soil salinity. The increased salinity can be explained by comparing the EC of biochar (2.85 dS/m) against that of soil prior to soybean planting (0.91 dS/m) (Fig. 5).

The highest EC was 1.46 (dS/m) in the 16-ton biochar treatment with non-inoculated bio-fertilizer, whereas the lowest electrical conductivity was 1.02 (dS/m) in the biochar-free treatment with inoculated bio-fertilizer. Given that saline soils are attributed to EC higher than 4 dS/m, the treatments in this study led to no variations in salinity.

Yield traits

Yield traits that have been measured include pod number per plant, grain yield, harvest index, oil content, and protein content that we discuss separately (Table 6).

Pod number per plant

The comparison of mean value for interaction of bio-fertilizer in biochar on the number of pods per plant revealed that the highest number of pods per plant (49) was achieved in the 16-ton biochar treatment with inoculated bio-fertilizer, while the lowest number of pods per plant (Rowell 1994) was observed
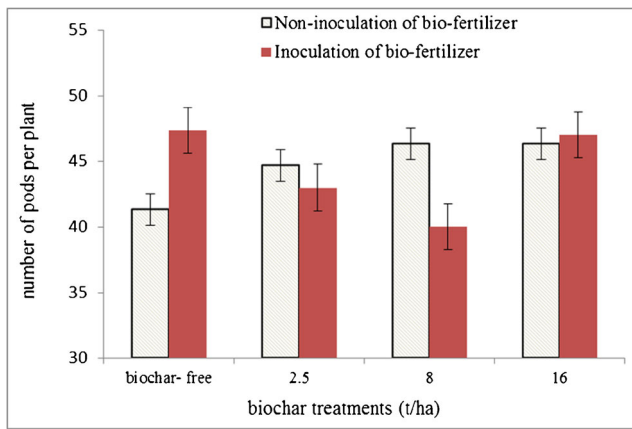
Table 6 Comparison of mean values for the effects of different biochar and bio-fertilizer levels on the traits

Treatment	Number of pods per plant	Yield (kg/ha)	Harvest index (%)	Oil content	Protein content
Biochar (t/ha)					
Biochar-free	38 [c]	2335 [d]	0.51 [d]	15.3 [b]	40.6 [a]
2.5	45 [b]	3347 [b]	0.54 [b]	16.0 [b]	38.8 [bc]
8	47 [a]	3417 [a]	0.57 [a]	17.5 [a]	37.1 [c]
16	47 [a]	3292 [c]	0.53 [c]	16.0 [b]	39.8 [ab]
Bio-fertilizer					
Non-inoculation of bio-fertilizer	44 [a]	3086 [a]	0.54 [a]	16.0 [a]	39.5 [a]
Inoculation of bio-fertilizer	45 [b]	3110 [a]	0.54 [a]	16.4 [a]	38.6 [a]

Mean values with the same letters in each column indicated no statistically significant differences

Fig. 6 Interaction of bio-fertilizer in biochar on the number of pods per plant. Each value is the average of 3 replicates. Vertical bars indicate +/- standard error. Different letters indicate a significant difference (P<0.05)

in the biochar-free treatment with inoculated bio-fertilizer. The difference between the highest and lowest number of pods is 32% (Fig. 6). This might be due to the fact that soil amelioration and crops supplied with adequate nutrients have more vegetative growth, longer linear growth rate, and more dry matter accumulation which is directly related to an increment in pod number.

The results were consistent with those obtained by Bayan who examined the effect of biochar on growth and nodulation of soybeans leading to 62% increase in the number of soybean pods per plant under the 2% wheat straw biochar treatment compared to the biochar-free treatment (2013). The number of pods per plant is one of the most important components of grain yield, making up the largest contribution in variation of grain yield (Caliskan et al. 2007).

Grain yield

The comparison of mean values revealed that the highest yield was 3440 kg/ha in the 8-ton biochar treatment with inoculated
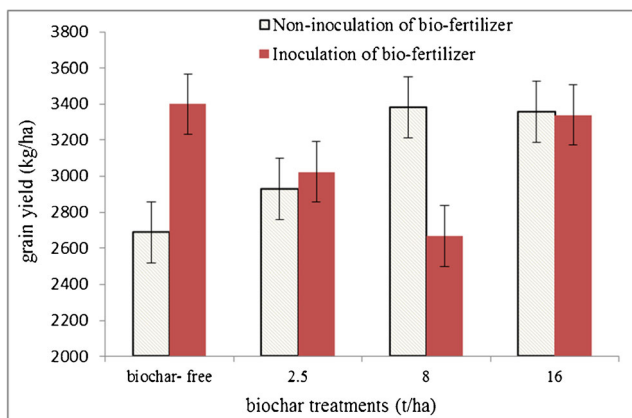

Fig. 7 The interaction of bio-fertilizer in biochar on grain yield. Each value is the average of 3 replicates. Vertical bars indicate +/- standard error. Different letters indicate a significant difference (P<0.05)
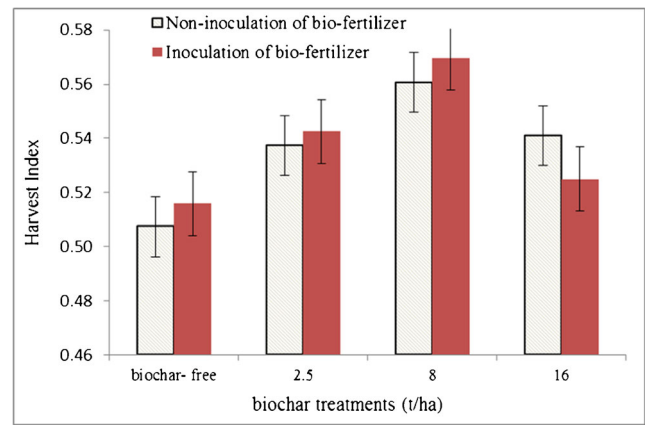

Fig. 8 The interaction of bio-fertilizer and biochar on harvest index. Each value is the average of 3 replicates. Vertical bars indicate +/- standard error. Different letters indicate a significant difference (P<0.05)

bio-fertilizer, whereas the lowest yield was 2280 kg/ha in the biochar-free treatment with non-inoculated bio-fertilizer (Fig. 7). The difference between the highest and lowest grain yields is 51%. This could be attributed to reclaim in the level of soil acidity from application of biochar and in turn increases the number of pods per plant and hence grain yield.

The effect of biochar and soluble fertilizers on wheat yield has been studied (Solaiman et al. 2010). They found that the 6-ton biochar treatment improved grain yield by 18% in Australia. The findings were consistent with those of the current study.

Biederman and Harpole argued that one of the main practical advantages of biochar is to enhance grain yield and reduce the leaching of soil nutrients (2012). Soil was amended with biochar, compost, and their mixture at field level (Agegnehu et al. 2016). They found that maize grain yield was significantly increased by 10–29% by organic amendments, which is consistent with the findings of this study. As residual nitrogen and cation exchange capacity increase in the soil, greater nutrients are provided to soybean, thus enhancing grain yield.

Harvest index

The highest harvest index in this research was 56.90% in the 8-ton biochar treatment with inoculated bio-fertilizer, whereas the lowest harvest index was 50.70% in the biochar-free treatment with non-inoculated bio-fertilizer (Fig. 8).

For 4 years, Major examined the effect of biochar on corn farming across Bogotá savanna, Colombia (2010). The results showed that addition of 20 tons per hectare of biochar in 2003 to 2005 improved the corn harvest index from 44 to 50%.

Oil content

The results of ANOVA indicated that the effect of biochar treatments on oil content was significant ($p \leq 0.01$). The highest oil content was 17.50% at 8-ton biochar treatment, while the lowest oil content was 15.33% at biochar-free treatment. The difference between the highest and lowest oil content was 2.17%. In a study on the effect of mycorrhizal fungi and growth-promoting bacteria (PGPR) on yield, yield components, and sesame oil content at different phosphor levels, Yavarpour reported that the PGPR-containing bacteria increased oil content in sesame even though it was statistically insignificant (2013). Consistent with the findings of this study, he indicated the incremental effect of bio-fertilizers on the oil content in different plants. Shehata and Khawas examined the effect of bio-fertilizer on growth parameters, yield, and yield components of sunflower (2003). They found that PGPR-containing bio-fertilizer improved yield and oil content in sunflower. Table 5 displays the correlation coefficients, where there is a positive correlation between oil content with grain yield ($r = 0.54^{**}$) and harvest index ($r = 0.74^{**}$). Also there is a positive correlation between oil content and the number of pods per plant ($r = 0.45^{*}$). Furthermore, there is a negative correlation between oil content and protein content ($r = -0.95^{**}$). The oil content is strongly influenced by environmental conditions. It has been found that oil content and protein content are inversely correlated (Mertz-Henning et al. 2018).

Protein content

The results of ANOVA indicated that the effect of biochar treatments on protein content was significant ($p \leq 0.01$) (Table 3). The effect of bio-fertilizer and interaction of bio-fertilizer in biochar on protein content was not significant. The comparison of mean values for the effects of biochar on protein content indicated that the biochar treatment reduced the protein content. The highest protein content was 40.58% in the biochar-free treatment, while the lowest protein content was 37.09% in the 8-ton biochar treatment (Table 4). The difference between the highest and lowest protein content was 3.49%. The results indicated a negative correlation between oil content and protein content, as highlighted by numerous researchers (Aliari 2000). The correlation coefficients (Table 5) indicated a negative correlation between protein content and harvest index ($r = -0.70^{**}$), grain yield ($r = -0.53^{**}$), oil content ($r = -0.95^{**}$), and thousand grain weight ($r = -0.42^{*}$).

Conclusions

According to the results, the application of 8-ton biochar is preferred over other levels. Moreover, the combined bio-fertilizer left no beneficial effect on growth and yield of soybean in the study area.

Given the results of soil tests before planting and after harvesting soybean, it is recommended to apply biochar mainly in soils with a low pH. This in turn will increase the soil acidity to absorb nutrients in the plant more easily. The cation exchange capacity increased by the biochar treatment leads to greater exchange of cations required by the plant, while contributing to higher grain yield. Although it had a slight effect on electrical conductivity, biochar is not recommended for saline soils.

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