Journal of Plant Sciences
2022; 10(5): 165-176
http://www.sciencepublishinggroup.com/j/jps
doi: 10.11648/j.jps.20221005.11
ISSN: 2331-0723 (Print); ISSN: 2331-0731 (Online)

Effects of Biochar and Rhizobium Inoculation on Growth, Yield and Yield Components of Common Bean at Jimma, Southwestern Ethiopia

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To cite this article:
Abdulkedir Bediru Shekedir. Effects of Biochar and Rhizobium Inoculation on Growth, Yield and Yield Components of Common Bean at Jimma, Southwestern Ethiopia. *American Journal of Agriculture and Forestry*. Vol. 10, No. 5, 2022, pp. 165-176.
doi: 10.11648/j.jps.20221005.11

Received: August 18, 2022; **Accepted**: September 13, 2022; **Published**: October 11, 2022

Abstract: Common bean (*Phaseolus vulgaris* L.) is a vital grain legume tremendously prioritized for meals and the marketplace in Ethiopia. However, the productiveness of common bean is low at country-wide in addition to zonal tiers. Poor soil fertility, due to soil acidity, is one of the factors that affect the growth and yield of beans. The low productivity of beans is likewise associated with decreased nodulation in acid soils. The use of Rhizobium inoculation (RI) may improve nodulation but nutrient uptake by the crop under acidic conditions is very low. Biochar (BC) has been reported to be of potential value in improving soil properties and in reducing the harmful effects caused by soil acidity. Therefore, an experiment was conducted to determine the effects of BC and RI on growth, yield, and yield component of common bean at Jimma under lath house conditions. The treatments consisted of three levels of BC (0, 5, and 10 t ha^{-1}) and two levels of RI (RI1 and RI0). The experiment was laid out in RCBD with four replications. Results revealed that combined application of BC and RI highly significantly ($P < 0.01$) influenced the number of nodules, nodule dry weight, root dry weight, root volume, number of pods, and seed yield and significantly ($p < 0.05$) influenced days to physiological maturity, number of effective nodules, nodule volume, pod length and number of seed per pods. Combined application of 10 ton ha^{-1} BC with RI1 increased pod number and seed yield over sole applications of RI, and when compared to the control. In general, the results of this study indicated that the combined application of BC and RI1 has a promising positive effect on yield and yield components of common bean. Hence, the combined application of 10 tons of BC ha^{-1} with RI1 resulted in maximum seed yield and yield components of common bean. However, since the data was obtained from lath house conditions, it will be worth repeating the experiment under field conditions but care has to be taken upon transferring the result, to draw sound conclusions and recommendations.

Keywords: Common Bean, Bio Char, Rhizobium Inoculation, Soil Acidity

1. Introduction

Common bean (*Phaseolus vulgaris* L.) belongs to the Fabaceae own family. The common bean is one of the principal legumes which has been consumed worldwide for its edible seeds and pods. It originated from South America and has grown as a cheap source of protein in the world and the majority of Sub-Saharan African people [1]. Farmers frequently use it as a source of food and component in crop rotation due to its ability to fix atmospheric nitrogen [2].

According to [3], the total global production of about 25 million MT with a productivity of 792 kg/ha out of which about 6 million MT is produced in Africa [4]. Sub-Saharan Africa and Latin America are the leading cultivators of common beans. Common bean is also produced in many parts of the tropics and sub-tropics as well as at some points of the temperate areas with approximate contributions of three-quarters of the global [5].

In Ethiopia, the common bean is highly preferred by Ethiopian farmers because it is the most important food legume and source of protein [6]. Moreover, it has fast maturing characteristics that enable households to get the cash income required to purchase food and other household needs when other crops have not yet matured [7]. The economic significance of the common bean in Ethiopia is quite considerable since it represents one of the major food and cash crops [8]. It has an incredible capability for the country as it has been duly recognized by many researchers and organizations for its economic significance and it uses diverse at-home demands.

Common bean is among the top-ranking pulses in terms of area coverage, with an increasing trend for the last few years [9]. Common bean is produced in different agro-ecological zones in Ethiopian low and mid-altitude areas [10] on 0.37 million hectares of area coverage with a total annual production of 0.51million MT [3]. According to [9] in terms of the geographical distribution of production, Oromia shares 51% of common bean production and SNNPR contributes 27% of the country's common bean production, followed by Amhara (20%) and, Benishangul-Gumuz (1%) and 1% contributed from the other regions. However, the countrywide common bean average yield (1.59 t ha^{-1}) [9] is by far below the average yield reported at research sites (2.5 to 3.0 t ha^{-1}) [11].

In the Jimma zone, area coverage of the crop was about 4906.3 ha with a total production of 4,428.6 tones and productivity of 0.9 t ha^{-1} [9] which, in turn, is far below the national average yield. The low national average yield and the zonal low yield may be attributed to a combination of several production constraints. Among others, lack of soil fertility management and declined soil nutrient availability associated with low pH of the soils are among the tops. More importantly, the major common bean-producing areas in South Western Ethiopia are characterized by high rainfall and acidic soils [12]. In addition, farmers have limited capacity to purchase and apply commercial fertilizers, which is the principal cause of the very low productivity of beans in Ethiopia.

The use of Rhizobium-legume symbiosis is a cheap and usually more effective agronomic practice for ensuring an adequate supply of N for legume-based crop production than an application of fertilizer nitrogen for resource-poor farmers. However, the common bean is one of the poor N fixers among legumes, especially under acid soils where nodule formation limits crop growth. Inoculation of seeds with commercial strains Rhizobium has been suggested to improve the nodulation of the crop. But, the activity of Rhizobium under acidic conditions is very limited and nodulation could be hampered to a larger extent due to soil pH affecting the activity of microorganisms according to [13], indicating that the biological nitrogen fixation in a very acidic soil (pH 4.4) can be reduced up to 30%. The activity of Rhizobium bacteria is therefore considered as the first limiting plant nutrient for bean yield in acidic soils.

Tang C., et al., 2013 [14], also reported that to date, various methods such as applications of lime and organic materials have been studied to determine their effectiveness in ameliorating soil acidity. The application of lime is believed to enhance soil health status by improving soil pH and enhancing microbial activity. In addition, organic wastes have been reported to increase acidic soil pH and correct acidity, mainly due to the neutralization of organic acid anions from organic wastes by decarboxylation [15].

However, caveats should be applied because surface applied lime moves slowly through soil profiles and consequently is an inefficient in ameliorating subsurface soil acidity [14]. Organic wastes also contain heavy metals and excessive N and P that may cause eutrophication [15]. In addition, unfinished organic wastes may still contain pests and diseases that are harmful to the target crop [16]. Therefore, looking for other options to tackle on enhancing plant growth under acidic conditions and nodulation for better N fixation and agronomic performance of the crop calls for urgent investigation.

Biochar is therefore the other alternative that can be used as a cheap soil amendment option to boost soil fertility. Biochar is the by-product of the thermal decomposition of organic materials in an oxygen-limited environment [17]. It improves soil quality, such as by raising soil pH, increasing moisture holding capacity, attracting more beneficial microbes, improving cation exchange capacity, and retaining nutrients in soil [15]. It can be an effective and long-lasting amendment to ameliorate the soil environment [18]. Biochar is also considered to be much more effective than other organic matter sources in retaining and making nutrients available to plants [19]. However, most biochar materials, unless derived from manure or blended with nutrient-rich materials, do not substitute for conventional fertilizer, so adding biochar without necessary amounts of nitrogen, phosphorus and potassium should not be expected to provide improvements to crop yield [20].

Biochar application for legume crop production in acidic soils may increase nutrient availability, particularly P, contributing to crop yield and phosphorus deficiency. It also significantly impaired both host plant growth and symbiotic N fixation. This clearly indicates that N fixation has a higher phosphorus requirement for optimal functioning than that required for host plant growth. Likewise, Nitrate assimilation and nodule number, as well as total and specific nitrogenase activity, also increase with the addition of P. The potential of biochar as a soil amendment in agricultural fields has been recently recognized and yet it is not fully utilized and its role in combination with Rhizobium inoculation is poorly understood. Hence, the present research is proposed aiming at developing an optimum combination of biochar application along with Rhizobium inoculation.

Specific objectives

1) Determine the response of common bean to different levels of biochar and Rhizobium inoculation on growth, yield, and yield components.
2) To examine the interaction effects of Biochar and Rhizobium inoculation on response of common bean.

2. Research Methods

2.1. Description of the Experimental Site

The pot experiment become performed at Jimma

University College of Agriculture and Veterinary Medicine (JUCAVM) in 2017/18 under lath conditions for the reality that strong acid soil become wanted and taken from Jimma Agricultural Research Center (JARC). Jimma is geographically placed 346 km Southwest of Addis Ababa at an elevation of 1753 meters above sea level situated at a latitude of 70 S 42' 9"N and longitude 360 47' 6" E in Ethiopia. The experimental site receives an average annual rainfall of 1559 mm with maximum and minimum temperatures of 26.8 and 13.6°C, respectively. Soil type turned into silt clay, and more typically considerable vegetation turned into coffee, maize, banana, and sorghum.

2.2. Experimental Materials

Common bean variety, Nasir was obtained from Melkasa Agricultural Research Center (MARC). The variety was released by MARC and it is well adapted to study areas (Table 1). Triple Super Phosphate (TSP, 20% P) and Urea (46% N) were used as sources of P and starter N fertilizer, respectively. An effective Rhizobium strain (Rhizobium legume strain HB-429) was purchased from Menagesha Bio-fertilizers laboratory Private Limited Company, Menagesha, Ethiopia. Additionally, coffee husk biochar and plastic pots were used.

Table 1. *Descriptions of Nasir variety and its agronomic requirements.*

Agronomic Characteristics	Descriptions	Remark
Year of release	2003	MARC/EIAR
Altitude (m.a.s.l)	1200-1900	
Annual Rainfall (mm)	500-800	
Planting date	Mid-June-Early July	
Days of 50 flowering	40-55	
Days to 95% maturity	86-88	
Growth habit	Bushy	
Seed Colour	Red	
yield (ton ha^{-1})	2.5-3	research site

Source: [21].

2.3. Treatments and Experimental Design

The experiment had two factors namely three levels of biochar (BC1 = 0 t ha^{-1}, BC2= 5 t ha^{-1}, and BC3=10 t ha^{-1}) and Common bean seed which was not inoculated and inoculated with Rhizobium inoculation. Therefore, the treatment was arranged as 3×2 in factorial combinations in RCBD with four replications. Totally, there were 6 treatment combinations, which were randomly assigned to each pot.

Table 2. *Detail of Treatment Combinations.*

No.	Treatment	Combined rates of Bio char, and *Rhizobium* inoculation	Remark
1	$B1RI_1$	0ton of biochar, with inoculation	0ton BC+RI
2	$B2RI_1$	5ton of biochar, with inoculation	5ton BC+RI
3	$B3RI_1$	10ton of biochar, with inoculation	5ton BC+RI
4	$B1RI_0$	0ton of biochar, without inoculation	0ton BC+R I_0 Control
5	$B2RI_0$	5ton of biochar, without inoculation	5ton BC + RI_0
6	$B3RI_0$	10ton of biochar, without inoculation	10ton BC +RI_0

Where BC1, BC2, and BC3 are 0-ton per hectare biochar, 5ton per hectare biochar, and 10-ton per hectare biochar respectively and RI0 Seed is not inoculated and RI1 seed is inoculated with Rhizobium respectively.

2.4. Experimental Procedure and Management

2.4.1. Preparation of Biochar and Application

The biochar was delivered from a locally accessible Coffee husk material from Coffee processing sites. The coffee husk was pyrolyzed at a temperature of 500°C with 3 hours of residence time as proposed by [22, 23]. After the pyrolysis, it was watered to chill off, air dried, put into sacks, took required sums, grounded to little granules, and went through a 2 mm sifter to have a similar molecule size as that of the soil [23]. At long last, it was subjected to laboratory analysis to determine some other selected chemical properties.

2.4.2. Lath House Activities

The trial was led with 24 pots (the absolute treatment pots were twelve, however, repeated each once to get an adequate number of plants for testing and supplanting the missed plants and put each pot one next to the other which had a similar treatment) and each pot was labeled for distinguishing proof reason. Since the study incorporates Rhizobium inoculation treatment, the current study was directed as a pot experiment. Hence, a sum of 24 plastic pots of eight kg weight with upper and lower (34 cm) surface width and level (36 cm) were filled up with eight kg of dry soil with various levels of biochar before planting. Before the pots were loaded up with soil, the soil was air dried for 72 hours, crushed finely, and weighted as required. The rates of biochar were calculated based on the bulk density method which is:

$$\text{Bulk density } (\rho b) = \frac{\text{Mass of the dry soil (g)}}{\text{The volume of the soil sample } (m^3)}$$

Source: [24].

Bulk density (ρb) was registered by the core sampler strategy which has both length and diameter of 30cm. Then, at that point, the soil in the core was taken out and dried to work out the mass of the dry soil (g) are volume of the soil sample (m^3) was computed by the formula of V=πr^(2) * L2. At last, coffee husk biochar was applied to the pots according to the rate for every treatment and mixed in with the soil equally before seedling activity. Nitrogen fertilizer in the form of urea (46% N) was applied as a starter dose at the recommended rate of 20 kg N ha^{-1} as recommended to include a small amount of nitrogen in the fertilizer of legume crops at sowing time to ensure the young seeding to have an adequate N supply until the Rhizobium strain can be functional. The spacing among replication and pots were 0.5 m and 0.25 m, respectively. Four seeds for each pot were planted at the suggested establishing depth of 6cm and afterward thinned to three plants after 15 days from planting. All other management practices were done as per the recommendations.

Carrier-based inoculants of strain were applied at the rate of 10 g inoculants for every kg of seed [25]. The inoculants were mixed with sugar with the addition of water to work with the adhesion of the strain on the seed. To ensure that the applied inoculants adhere to the seed, the expected amounts of inoculants were suspended in 1:1 proportion in a 10% sugar arrangement. The thick slurry of the inoculants was tenderly mixed in with the dry seeds so every one of the seeds got a thin coating of the inoculants. To keep up with the practicality of the cells, inoculation was done under the shade and allowed to air dry for 30 minutes, and planted in pre-arranged pots in two hours or less. Seeds were immediately covered with soil after sowing to avoid the death of bacterial cells. A pot with un-inoculated with inoculants seeds was planted first to avoid contamination.

2.5. Soil and Biochar Sampling and Analysis

The soil for the present study was collected from Jimma Agricultural Research Center (JARC) from a site whose pH has been determined to be strongly acidic (Table 3). Five soil samples were collected in a diagonal pattern from a depth of 0 to 30 cm by using an auger and bulked to obtain one representative sample. The sample was air dried, ground using a pestle and mortar, and allowed to pass through a 2 mm sieve. Before the commencement of the experiment, the soil sample (1kg) and the biochar were analyzed for physical and chemical properties using standard laboratory procedures at Jimma Agricultural Research Center Soil and Plant tissue chemistry Laboratory.

Soil pH was resolved potentiometrically using a pH meter with a combined glass electrode in a 1:2.5 soil to water supernatant suspension [26, 27] technique was utilized to decide the organic carbon content and organic matter substance. The total nitrogen of the trial site soils was 0.14%. As per [28], the nitrogen in the soil is low (Table 3). It was determined by the Kjeldahl technique using a micro-Kjeldahl distillation unit and Kjeldahl processing stand as reported by [29]. Available soil phosphorus was extracted by the Bray II strategy [30] and decided colorimetrically by spectrophotometer as per [31], the trial soils were viewed as exceptionally low in P (3.53 ppm) and lacking in phosphorus as the area gets heavy rainfall; phosphorus is most likely fixed by high groupings of iron and aluminum. Cation exchange capacity (CEC) of the soil was determined by 1M ammonium acetate (NH_4OAc) saturated sample at pH 7 [31] where the standard paste was refined to gauge the ammonium freed by titration with acid and evaluated as moderate as per rating of [32]. The textural class of the soil is sandy clay having compositions of 44% clay, 52% silt, and 4% sand.

Biochar organic carbon content was determined by the Walkley-Black method and total nitrogen (TN) by the Kjeldahl method as cited in [33]. Available phosphorous (Av. P) was determined by using the Olsen extraction method [34] as cited in [23]. Cation exchange capacity was determined at soil pH 7 after displacement by using the 1N ammonium acetate method and then estimated titrimetrically by distillation of ammonium that was displaced by sodium [35].

After harvesting the crop, soil samples were taken from each pot. Then, a composite sample was prepared for analysis, and finally, the soil samples were taken to the Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) soil laboratory following the above procedures and analyzed to the determination of the effect of Biochar, phosphorus, and Rhizobium inoculation application on organic carbon, cation exchange capacity, soil available phosphorus and soil pH change on pot basis (Table 3).

Table 3. *Initial physicochemical properties of soil and biochar.*

Parameters	Soil		Biochar
	Values	Rating for soil Reference	
Soil Chemical			
pH	4.85	Strongly acidic [36]	10.13
OC%	2.14	High [32]	24.8
CEC (cmol)	14.78meq/100gsoil	Low [32]	78.05
TN%	0.14%	Low [28]	2.14
Av. P (ppm)	3.53	Very low [28]	12.87
Soil Texture			
Clay	44%		
Sand	52%		
Silt	4%		
Class	Sand clay		

Where Cmol =Cent mole, pH = hydrogen power,%OC = percent of organic carbon,%TN = Percent of total nitrogen, Av. P. ppm = available phosphorus in parts per million, and CEC = Cation exchange capacity.

2.6. Data Collected

2.6.1. Phenological and Growth Parameters

Days to flowering (DF):- The numbers of days from the date of planting to the date whilst approximately all of the plant life according to pot reached flowering level were recorded.

Days to physiological maturity (DM): Days to physiological maturity was recorded as the number of days from sowing to the stage when 90% of the plants in a pot have reached physiological maturity, i.e. the stage at which pots have mature pods in their upper parts with pods in the lower parts of the plants turning yellow. The yellowness and drying of leaves were used as an indication of physiological maturity.

Plant height (PH) (cm): Length of the central axis of the stem measured from the soil surface up to the tip of the stem of the plant at the time of physiological maturity and expressed as an average of three plants per pot.

2.6.2. Plant Root Characteristic

Taproot length (TL) (cm): was determined by measuring the central tap root using the ruler.

Root dry weight (RDW) (g): Dry weight of the root was recorded by taking all roots from three plants and put in the oven for 48 hours at 70°C to dry to a constant weight and finally it was converted to the dry weight of root per plant.

Root volume per plant (RV) (ml): Recorded as the average of three carefully up-rooted plant roots washed, dried in an oven at 70°C for 2 days, and measured using a cylinder graduated in ml.

2.6.3. Root Nodule Characteristic

The total number of nodules per plant (NNP): Three plants out of six had been sampled randomly from each pot at mid-flowering. The whole plant has cautiously uprooted the usage of a fork if you want to acquire intact roots and nodules for nodulation parameters. Uprooting was done by exposing the whole root system to avoid loss of nodules. The adhering soil was removed by soaking the ball of soil and root in a plastic pot filled with water and thoroughly rinsed in separate water-filled. Nodules from roots were removed carefully. The total number of nodules was counted and the average value of three plants was recorded as the number of nodules per plant.

The effective number of nodules (ENN): Nodules were dissected with the blade to observe their color in the center and the color nodules having pink or dark red centers due to the presence of leghemoglobin were recorded and the nodules having color white/green were considered ineffective nodules.

Nodules' dry weight (NDW) (g): was determined by placing nodules in an air-forced dryer at 70°C until a constant weight was obtained (48h).

Nodules volume per plant (NV) (ml): Recorded as the average of three carefully separated plant nodules washed, dried in an oven at 70°C for 2 days, and measured using a cylinder graduated in ml.

2.6.4. Yield and Yield Components

Number of pods per plant (PNP): by counting pods of three plants were taken from each pot at harvesting stage and the means were recorded as number of pods per plant.

Pod length (PL) (cm): it the distance between two ends of the pod and it was measured from seven selected pod per pot using ruler.

Number of seeds per pod (NSP): the total number of seeds on a plant divided by the number of pods on a plant after the pod was threshed.

Seed yield (SY) (g plant per 1): Three plants were threshed to determine seed yield. Finally, the average yield was reported in g per plant.

2.7. Data Analysis

All the data were examined for ANOVA assumptions. Then, those data were subjected to analysis of variance using SAS version 9.3 (SAS Institute Inc. 2012).

3. Results and Discussion

3.1. Effects of Combined Application of Biochar, and Rhizobium Inoculation on Phenological and Growth Parameters of Common Bean

3.1.1. Days to Flowering

The maximum period required to attain flowering (82.25days) was recorded from the combined application of 10ton ha^{-1} of BC with RI1. The shortest duration to flowering (74 days) was recorded for the control treatment (0 ton^{-1} BC + without inoculation (RI0)). This result was also statistically similar to the application of 10 and 5-ton ha^{-1} BC without inoculation (RI0) (Table 4).

Application of 10 ton ha^{-1} BC with RI1 increased the number of days to attained flowering by five in comparison to application of BC without RI1, and by eight days as compared to the control. Also, application RI1 without BC increased the number of days to reach flowering by four compared to the control treatment.

The days to flowering were delayed with an increment of the combined application rate of BC and RI1 which may be due to the delaying impact of nutrients acquired from this mixed application. Moreover, the significant difference among the various treatments is probably attributed to the traits of BC to retain carried out nutrients in the root zone of plants, enhance soil organic community abundance and capabilities that increase the amount of nitrogen which is more suitable for vegetative growth of the crop and prolonged days required to attain of flowering.

In line with this, [37] indicated that biochar application to the soil is an opportunity to enhance the hobby of Rhizobium bacteria thereby enhancing the efficiency of nitrogenous fertilizers which are effortlessly taken up by way of plant life for immediate vegetative growth, which ends up in longer photosynthetic apparatus via not on time flowering days.

It is likewise evident from the results that pots planted without Rhizobium inoculation had the shortest days to flowering. It became observed that with Rhizobium inoculation, the crop is behind schedule for the flowering initiation because of the prolonged boom length that is proved from the control pots, wherein the flowering changed into found in advance than the pots obtained Rhizobium. This is probably because of the supply of nitrogen via symbiotic nitrogen fixation that could have prolonged plant growth so that flowering and fruit set does not start early. The current finding is in agreement with [38, 39] who stated that soybean seeds inoculated with Rhizobium took most days to first flower over uninoculated control. Similarly, [40] observed that inoculation triggered overdue flowering in soybean in both glasshouse and field experiments.

3.1.2. Days to Physiological Maturity

The earliest days (83.50 days) of physiological maturity was recorded from control treatment while the longest days (93 days) to physiological maturity was recorded in the plants treated with 10ton ha^{-1} BC and inoculated with RI and it was statistically at par with 5 ton ha^{-1} BC with inoculated treatments (Table 4).

The delayed maturity might be due to Biochar multiplied the supply of soil nutrients and advanced soil physical properties together with bulk density and porosity thereby, enhanced root growth that could have more suitable the capability of flowers to access nutrients.

Then this in turn elevated amount of nutrients with in a plant that enhances leaf area expansion and greater leaf number developments that that prolonged the photosynthetic performance of plant at the same time as with Rhizobium inoculation might be because of the fact that higher nitrogen produced by way of N fixation through inoculation promoted vegetative growth which there by using prolonged days to maturity. In line with this result, [41] observed that significant effect of the different types of biochar on the maturity date of common bean plants.

3.1.3. Plant Height (PH)

The highest number of plant heights (59.75cm) was obtained from plants treated with 0ton ha^{-1} BC inoculated with Rhizobium inoculation. This result was statistically similar to the treatment received 5ton ha^{-1} BC but without Rhizobium inoculation, whereas the lowest number of plant height (47.48cm) was recorded from 0ton ha^{-1} BC + RI0 plants (Table 4).

Combined application of Biochar with Rhizobium inoculation ended in the maximum average number of plant height. The highest number of plant heights (59.46cm) was obtained from plants treated with 10ha^{-1} BC and not inoculated treatments. This end result become statistically similar to the application of 5ton ha^{-1} BC and not inoculated with Rhizobium and sole applications of Rhizobium inoculation (0ton ha^{-1} BC + RI) whereas the lowest number of plant height (47.48cm) was recorded from 0ton ha^{-1} BC + RI0 plants (Table 4).

The significant improvement in plant height is an indication of BC is more suitable for Rhizobium inoculation in the biological fixing of nitrogen that promotes the formation of chlorophyll which in turn resulted in higher photosynthetic activity, vigorous vegetative growth, and taller plants over the control. In addition to this, Rhizobium inoculation may have the potential to increase promoting substances (phytohormones) and the capability to solubilize phosphate that isn't always available for plant use on top of fixing N.

In conformity with this result, Rhizobium inoculation in cowpea significantly improved the plant height measured at four, six, and eight weeks after planting in both screen house and field experiments [42].

Table 4. *Effects of Bio-char and Rhizobium inoculation on Days to flowering (DF), Days to Physiological maturity (DM) and Plant height (PH) during the 2017/2018 growing season at Jimma, Southwestern Ethiopia.*

Factors		Parameters		
BC	RI	FD	MD	PH
0	I_1	78.00b^{cde}	87.75^{b}	58.37^{ab}
5	I_1	79.50^{b}	89.75^{ab}	52.87^{def}
10	I_1	82.25^{a}	93.00^{a}	53.44^{cdef}
0	I_0	74.00^{f}	83.50^{c}	47.48^{h}
5	I_0	77.50^{def}	88.25^{b}	58.46^{ab}
10	I_0	77.75^{def}	88.75^{b}	59.75^{a}
LSD (5%)		1.58	3.27	1.905
CV (%)		2.91	1.01	5.49

Means in the column followed by the same letter(s) are not significantly different at 5% level of significance. LSD (0.05) = Least significant difference at 5% level and CV (%) = coefficient of variation in percent.

3.2. Effects of Combined Application of Biochar and Rhizobium Inoculation on Root Nodule Characteristic

3.2.1. Number of Nodules Per Plant (NNP)

The highest number of nodules per plant (155.91) and the lowest number of nodules per plant (48.54) were observed from 10-ton ha^{-1} BC + Rhizobium and unfertilized or control, respectively (Table 5). The highest mean number of the nodule is also statistically on par with the pot received 5ton ha^{-1} BC + Rhizobium. Application of 10ton ha^{-1} BC + Rhizobium increased the number of nodules by 221.2%. Similarly, the application of Rhizobium without biochar increased the number of the nodule by 36.52% over the control.

There became low nodulation without BC even with Rhizobium inoculation, which suggests that soil acidity, is a hassle to rhizobial activity and nutrient availability. Hence, the increase in the number of nodules per plant might be attributed to the improved availability of nutrients due to the release of nutrients from the applied BC. This can be also associated with the fact that BC increases soil pH and stimulate the BNF capability of soil microorganism thereby enhancing root development and root nodulation. Application of BC with Rhizobium improves soil environmental conditions for the development of the Rhizobium population and Leucaena nodulation [42]. An increase in the number of nodules due to Rhizobium may be attributed to the inoculation of rhizobia, which increased the number of bacteria and therefore more nodules per plant were produced.

This result is in agreement with the findings of [42-44], who reported that the addition of biochar to soils, resulted in the average increase in nodule formation compared with control conditions.

3.2.2. Number of Effective Nodules (NEN)

Applications of 10-ton ha^{-1} biochar in combination with Rhizobium extended the number of effective nodules by 65.95% over the application of 0-ton ha^{-1} BC + Rhizobium and by 123.19% over the control treatment. Almost all the plants developed nodules on their roots but nodules in uninoculated plants were smaller in size.

This end result found that once inoculated with Rhizobium however without BC, minimal effective nodule number was recorded but increased with the combined application of BC with Rhizobium inoculation. The probable cause of the high number of effective nodules with BC application might be due to the improved soil properties that might stronger the root system which provides greater root development, root-soil contact, and root nodulation. Thus, it helps water absorption by increasing root growth and N nutrition by symbiosis. Moreover, Rhizobium inoculation increased root nodule numbers thereby, increased the number of effective nodules per plant.

Corroborating the result, [42] showed that 10 t/ha of biochar notably increased the range of powerful nodules (11.33) whilst compared with control (0t/ha). A similar finding was reported that [44, 45], the addition of biochar to soils resulted from an increased number of nodules per plant of groundnut in comparison with control conditions. Contrary to this, [43, 46] pronounced non-significant results of BC on powerful nodulation.

3.2.3. Nodules Dry Weight (NDW)

The maximum nodule dry weight (0.94g plant^{-1}) was recorded from pots treated with 10ton ha^{-1} BC + Rhizobium inoculation which is not statistically different from pots treated with 5ton ha^{-1} BC + Rhizobium inoculation (0.82 g plant^{-1}), whereas the lowest nodule dry weight (0.49g plant^{-1}) was obtained from control pots (without BC and Rhizobium inoculation) which is also statistically not difference with pots received Rhizobium inoculation alone (0ton ha^{-1} BC + Rhizobium inoculation) (Table 5).

When the crop was grown without BC, inoculation had no significant effect on nodule dry weight. This may be associated with the fact that BC increased the availability of essential nutrients such as P which has a direct role in root nodulation. Rhizobium bacteria are sensitive to soil acidity and require P, adequate soil moisture for their multiplication [47].

Likewise, [48, 49] mentioned that Rhizobium inoculation considerably affected the nodule dry weight of soybean together with starter N and common bean at different experimental locations respectively.

3.2.4. Nodule Volume (NV)

The lowest nodule volume per plant (0.96ml/plant) was recorded from the pots that did now not acquire BC rates and Rhizobium inoculation which is also likewise statistically similar to the application of 0ton ha^{-1} BC rate but inoculated with Rhizobium whereas the highest nodule volume per plant (2.06 ml/plant) changed into recorded from the combination of 10ton ha^{-1} BC and inoculation with Rhizobium. Application of 5-ton ha^{-1} biochar and Rhizobium also gave statistically the same result as did the former treatment combination.

The better nodule volume with BC might be due to essential soil nutrients consisting of phosphorous supplied by BC were enhanced the root development, an attachment of Rhizobium to root hairs, and root nodulation. Another reason could be the availability of P increased by BC is an essential ingredient for N- fixing bacteria and it has a big consequence on the development of a large number of nodules by providing the energy required for N fixation. Similarly, the highest nodule volume with Rhizobium might be due to inoculation of seeds with rhizobia increasing nodulation and nitrogen uptake which in turn increase nodule volume.

Table 5. *The number of nodules per plant (NNP), Number of effective nodules (NEN), Nodules dry weight (NDW), and Nodule volume (NV) as influenced by the application of BC, RI, and their interaction.*

Factors		Parameters			
BC	**RI**	**NNP**	**NEN**	**NDW**	**NV**
0	I_1	78.40^{cde}	52.11^{c}	0.56^{fg}	0.99^{bc}
5	I_1	154.29^{ba}	74.97^{ab}	0.82^{ab}	1.96^{ab}
10	I_1	155.91^{a}	81.53^{a}	0.94^{a}	2.06^{a}
0	I_0	68.54^{f}	36.23^{d}	0.49^{fgh}	0.96^{bc}
5	I_0	90.74^{cd}	52.75^{c}	0.70^{cd}	1.89^{b}
10	I_0	117.08^{bc}	66.98^{b}	0.72^{bc}	1.86^{b}
LSD (5%)		30.41	12.07	0.12	0.12
CV (%)		17.22	16.25	15.74	18.80

Means in the column followed by the same letter(s) are not significantly different at 5% level of significance. LSD (0.05) = Least significant difference at 5% level and CV (%) = coefficient of variation in percent.

3.3. Effects of Combined Application of Biochar and Rhizobium Inoculation on Plant Root Characteristics of Common Bean

The effect of BC and RI on root dry weight (RDW), tap root length (TL) and root volume (RV) are discussed as follows:

3.3.1. Root Dry Weight

The maximum mean for root dry weight (2.06g) turned into recorded from a combined application of 10 ton ha^{-1} of BC with Rhizobium inoculation. This result becomes statistically similar with the application of 5ton ha^{-1} BC + Rhizobium. While the minimum mean (1.18g) was recorded from the control (without biochar, and Rhizobium) treatment which was statistically in parity with the application of 0 ton ha^{-1} BC + inoculated with Rhizobium (Table 6).

The increase in root dry weight of common bean with combined application of biochar, and Rhizobium inoculation could be because of the positive impact of increased availability of nitrogen via more advantageous biological nitrogen fixation.

Similarly, inoculating Common beans with Rhizobium enhanced root development and also observed in this experiment greater values of root dry weight. This may be related to the development of plant roots and the growth of root hairs caused by Rhizobium. It is a well-documented fact that effective strains of Rhizobium with a higher ability to compete with native rhizobia not only form more nodules per plant but also enhance the dry weight of shoot and root [48]. Similarly, studies by [50] on chickpeas in the field and the glass house reported a significant increase in the dry weight of roots per plant by inoculating seeds with Rhizobium.

On the other hand, Biochar addition assists as a soil conditioner and will increase soil nutrient concentrations, surface area, and porosity, reducing soil greenhouse gas emissions and enhancing microbial activity leading to the development of plant root growth in turn increasing root dry weight. This result is in settlement with the findings of [41] suggested that the best root dry mass of common bean was obtained via growing concentrations of biochar addition to the soil when it comes to the control treatment.

3.3.2. Root Volume

The highest root volume consistent per plant (0.94ml) was recorded for the combined application of 5 tons ha^{-1} of BC with Rhizobium inoculation. This result was statistically similar to the root volume (0.93ml) obtained for the combined application of 10 tons ha^{-1} of BC with Rhizobium inoculation. On other hand, the lowest value for root volume (0.67ml) was obtained from the control treatment. This result was statistically similar to the pot fertilized with Rhizobium inoculation without BC (Table 6).

These results may be justified by improved soil chemical, physical, and biological properties due to BC application. BC with a porous structure decreases the soil bulk density, reduces mechanical resistance, and increased water retention potentially facilitating root proliferation which in turn increased root quantity. Similarly, Rhizobium inoculations are symbiotic bacteria that facilitate the formation of roots of legume hosts, thereby increasing root density and root extent. In line with this, [41, 51] reported that the greatest root volume increased root number or biomass and productivity of crops in soils fertilized with biochar.

3.3.3. Tap Root Length

Application of 5-ton ha^{-1} biochar in combination with Rhizobium inoculation resulted in maximum (19.5cm) tap root length. This result turned in to be statistically similar to the application of 10-ton ha^{-1} BC + Rhizobium inoculation. On other hand, the minimum mean value (12.39cm) was observed for the control treatment (Table 6).

BC may have positive outcomes on root length; t this is probably thru its ability to change the soil pH, decreases the soil bulk density, and reduces mechanical resistance, which hinders the growth and length of root to potentially facilitate root proliferation and improved root-soil contact. Similarly, Rhizobia inoculation is soil bacteria that induce root length on leguminous plants.

Corroborating this result, [52] reported that the responses of the tap root length to the presence of biochar are due to a decrease in both inorganic monomeric Al (mainly in Al^{2+} and Al^{3+}) and colloidal Al, and an increase in P. Similarly, [53, 44, 54] reported that BC may have positive effects on root length, through its potential of changing the soil pH and reducing the bulk density in soils leading to increase root penetration that allows the increment in length. Likewise, Rudresh (2012) reported the effects of Rhizobium inoculation in Vigna mungo and Vigna radiate and found that both V. mungo and V. radiata varieties inoculated plants possessed greater length and number of roots. [43] Also found that Rhizobium inoculation with groundnut increased root length.

Table 6. *Mean of effects of BC and Rhizobium inoculation (RI) application on Root dry weight (RDW), Root volume (RV) and, Tap Root length (TRL).*

Factors		Parameters		
BC	RI	RDW	RV	TRL
0	I_1	1.30^{de}	0.74^{ef}	15.05^{fg}
5	I_1	1.98^{ab}	0.94^{a}	19.5^{a}
10	I_1	2.06^{a}	0.93^{ab}	19.45^{ab}
0	I_0	1.18^{e}	0.67^{f}	12.39^{h}
5	I_0	1.63^{bcd}	1.18^{cd}	16.67^{cd}
10	I_0	1.88^{bc}	1.33^{c}	16.27^{cde}
LSD (5%)		0.43	0.17	2.18
CV (%)		18.04	10.87	8.90

Means in the column followed by the same letter(s) are not significantly different at 5% level of significance. LSD (0.05) = Least significant difference at 5% level and CV (%) = coefficient of variation in percent.

3.4. Effects of Combined Application of Bio Char and Rhizobium Inoculation on Yield and Yield Components of Common Bean

Combined application of BC and Rhizobium inoculation tested on the number of pods per plant (NPP), pod length (PL), the number of seeds per pod (NSP), and seed yield (SY) are discussed as follows:

3.4.1. Number of Pods Per Plant (PN)

The number of pods per plant, in the long run, determines the productive potential of the common bean which is the main yield component. The highest number of pods (19.55) per plant was obtained from the combined application of 10 ton ha^{-1} BC with Rhizobium inoculation which was statistically the same with 5 ton ha^{-1} BC + Rhizobium inoculation while the lowest value (8.42) was recorded for the control (0ton ha^{-1} BC + uninoculated seed) treatment, which was statistically identical with the treatment that includes Rhizobium inoculation without biochar (Table 6).

The elevated number of pods per plant with increasing BC levels might be because BC improves soil nutrient availability by reducing nutrient fixation in acid soils particularly enhancing Phosphorous availability within the soil which would have increased the intensity of photosynthesis, nitrogen fixation, root development, flowering, seed formation, and fruiting. Similarly, the positive consequences of the inoculants might be due to a better amount of nitrogen rendered through nitrogen fixation promoting vegetative growth and plant height and thus improving the number of pods per plant. In conformity with this result [41] said that the application of biochar with increasing doses undoubtedly altered the number of pods of common bean.

3.4.2. Pod Length

The highest (13.94cm) length of the pod resulted from 10ton ha^{-1} BC + Rhizobium inoculation (Table 7). On the other hand, the lowest (7.31cm) pod length was recorded from the control treatment. The pod length was increased with combined application of BC and Rhizobium inoculation compared to a lone of their application.

The possible reasons for the maximum pod length determined from the combined application of BC, and Rhizobium inoculation might be because of the quick response of common bean to the application of BC that enhanced soil nutrients to crop faster and eventually leached beyond the root zone of crops. It became also might be associated with the increased nutrients within the soil that led to higher vegetative growth which in turn allowed the crops to produce greater photo assimilated within the pods. BC addition to soil increased N fixation by both free-living and when supplemented with Rhizobium inoculation which is an alternative to the expensive inorganic nitrogen fertilizers that stimulated pod growth and length.

This result is in agreement with the works of [55] who stated that biochar additions at different levels drastically increased BNF by rhizobia at all application rates (30, 60, and 90 grams per kilogram of soil).

3.4.3. Number of Seeds Per Pod

The total number of seeds per pod was increased with combined application of BC, and Rhizobium inoculation compared to sole application of either BC or RI. The maximum (7.62) seeds per pod were recorded from the combined application of BC at a rate of 10 tons $ha^{-1}$and RI1 which was also statistically on par with 5ton ha^{-1} biochar + Rhizobium inoculation. On the alternative hand, the minimal range of seeds per pod (3.86) was recorded from the control (0ton ha^{-1} BC + uninoculated seed) treatment, which become also statistically on par with the number of seeds per pod acquired from 0ton ha^{-1} BC + RI1.

3.4.4. Seed Yield (g plant^{-1})

Among the various interactions, the highest seed yield (31.63 g $plant^{-1}$) was obtained from the combination of 10 ton ha^{-1} BC with Rhizobium inoculation and it increased by 83% over the control treatment followed by 5 ton ha^{-1} BC and Rhizobium inoculation that increased 80% over the control treatment (Table 7). In contrast, the lowest Seed yield (17.28g $plant^{-1}$) was recorded from treatment containing 0ton BC ha^{-1} with no Rhizobium inoculation which statistically at par mean seed yield to that of 0 ton BC ha^{-1} + RI1.

The increased Seed yield with increasing BC levels and Rhizobium inoculation might be because BC improves soil nutrients availability through the reduction in their fixation in acid soils as well as increases soil nutrient concentrations and microbial activity and enhanced root system due to favored more extensive soil exploration, nutrient mobilization, and uptake efficiency, and thus contribute to maximum yields.

The positive effects of BC on common bean productivity could be due to BC not only increasing the microbial activity but also increasing the mineralization of soil organic matter so that more crop nutrient needs were met. Although the sole application of BC (in the absence of the RI) increased common bean growth and yield compared with control (Table 7), these increases were lower than those caused by the combined application of BC and Rhizobium inoculation. These improvements in inoculated treatments could be accredited to improved biological nitrogen fixation by rhizobial inoculants which increased nitrogen supply to the plants and subsequently increased total leaf chlorophyll contents of the legumes [56] which in turn increased yield. [37] Also observed good outcomes on crop yield after biochar was applied and indicated that these outcomes are related to the enhancement of soil chemical properties and enhancement in nutrient availability to plants.

Table 7. *Mean of effects of BC, and Rhizobium inoculation (RI) application on Pod number (PN), pod length (PL), number of seed per pod uptake (NSP), and seed yield (SY) of common bean.*

Factors		Variables			
BC	RI	PN	PL	NSP	SY
0	I_1	8.52^{f}	8.98^{g}	4.51^{ef}	18.23^{fg}
5	I_1	17.71^{ab}	11.62^{bc}	6.65^{ab}	26.90^{bc}
10	I_1	19.55^{a}	13.94^{a}	7.62^{a}	31.63^{a}
0	I_0	8.42^{f}	7.31^{h}	3.86^{f}	17.28^{g}
5	I_0	11.69^{cde}	9.52^{efg}	5.49^{ecd}	23.14^{cde}
10	I_0	11.72^{cde}	10.42^{de}	6.33^{bc}	23.48^{cde}
LSD (5%)		2.33	1.27	1.04	4.63
CV (%)		13.09	8.03	12.74	12.63

Means in the column followed by the same letter(s) are not significantly different at 5% level of significance. LSD (0.05) = Least significant difference at 5% level and CV (%) = coefficient of variation in percent.

4. Conclusion

he results of this study confirmed that growth, yield, and yield component parameters of common bean such as phonological and growth, nodule characteristics, root characteristics, yield, and yield components of common bean were increased along with increased application rates of BC and RI. Accordingly, the interaction effect of rates of BC and Rhizobium inoculation significantly influenced on Days to flowering, Days to physiological maturity, plant height, the total number of nodules per plant, number of effective nodules, nodule dry weight, nodule volume, taproot length, root dry weight, root volume and number of pods per plant. The interaction effect of BC rates and RI significantly influenced root dry weight, root volume; taproot length, the total number of nodules per plant, nodule dry weight, and grain yield of common bean.

In conclusion, results of the present study indicated that the combined application of 10 ton ha^{-1} BC and RI1 substantially improved growth, yield, and yield components of common bean, and accordingly all the combinations of BC levels and RI1 showed growth and related yield parameters as compared to the control treatment.

5. Recommendation

However, the current investigation was done lath-house, it is suggested that the effect of biochar in combination with RI should be tested in the field, but maximum care needs to be taken when we transfer results of a pot experiment to the field as root to soil volume varies on pot Vs field to come up with comprehensive recommendation rates for biochar and RI.

Therefore, future research works should focus on:-

1) Identifying and Comparing different types of materials (feedstock) used for the preparation of biochar for soil amendment to provide maximum yield,
2) Identifying locally available pyrolyzing mechanisms for biochar preparation,
3) Evaluating the effect of combined application of biochar, and RI using different varieties of common bean and
4) Testing the effect of biochar with a rate of greater than 10 ton ha^{-1} with the combination of RI.

List of Abbreviations and Acronyms

BC	Biochar
BNF	Biological Nitrogen Fixation
CSA	Central Statistical Agency
JUCAVM	Jimma University College of Agriculture and Veterinary Medicine
M AS L	Meter above Sea Level
MARC	Melkasa Agricultural Research Center
MoA	Ministry of Agriculture
PSB	Phosphorus solubilizing bacteria
RI	Rhizobium Inoculation
SNNPR	South Nation and Nationality People Representative

Acknowledgements

I gratefully acknowledge my major advisor to Prof. Amsalu Nebiyu and Co-advisor Dr. Milkiyas Ahmed for their whole-hearted guidance and constructive comments at all stages of the research work and thesis write-up. A lot of thanks to Sekoru Woreda for supporting my M. Sc. studies and granting me a full scholarship. I would like to express my sincere gratitude to my Father Bediru Shekedir, and my Mother Amberu Hajji Yasin, for their strong support throughout my study.

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