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Impact of addition of biochar along with *Bacillus* sp. on growth and yield of French beans

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ABSTRACT

Use of chemical fertilizers to enhance crop yield may lead to pollution, acidification or mineral depletion in soil. Plant growth promoting rhizobacteria (PGPR) are an alternative to chemical fertilizers as they contribute towards promotion of plant growth and yield of different crops. But if added with biochar, they result not only in enhancement of crop yields, but also help in preventing fertilizer run-off, leaching, retaining moisture and helping plants through periods of drought. To the best of our knowledge this is first report to study the effect of biochar along with a potential PGPR strain, *Bacillus* sp.

A pot experiment was done with 6 different treatments viz. pure soil, soil + biochar, soil + *Bacillus* sp., soil + biochar + *Bacillus* sp., soil + biochar + commercial biofertilizer (Biozyme), and soil + chemical fertilizer (Di-ammonium phosphate, DAP), and the length and biomass of root and shoot, seed yield and nutrient uptake were measured in French beans (*Phaseolus vulgaris*). Generally, all treatments showed a significant increase in growth and yield as compared to plants grown in untreated soil. It was observed that addition of biochar to soil influenced the overall growth of plants positively but the inoculation with *Bacillus* sp. or Biozyme enhanced this effect further. The treatment, soil + biochar + *Bacillus* sp. also showed the highest number of phosphate solubilizing bacteria in the rhizosphere of plants and percent N content in shoots, whereas the highest P content was observed in soil + DAP, followed by soil + biochar + *Bacillus* sp. combination. Hence, it can be concluded that both biochar and the bioinoculant, *Bacillus* sp. are good treatments for sustainable agriculture.

1. Introduction

The extensive use, rather misuse of chemical fertilizers has led to the deterioration of the environment causing innumerable problems (Savci, 2012). Chemical fertilizers not only contaminate the water (Shamrukh et al., 2001) but also degrade soil fertility in the long run (Chen et al., 2009; Li et al., 2010). At global and local level there is growing consciousness to protect the environment without compromising sustainable agriculture. Organic farming works in accord with nature. This involves using techniques to achieve high crop yields without harming the natural environment or the people who live and work in it.

Biochar is a stable solid material obtained from the carbonization of biomass and can endure in soil for thousands of years. It is also known as Amazonian dark earth or Indian black earth or *terra preta de Indio*. It is characterized by the presence of low-temperature charcoal in high concentrations; of high quantities of pottery shards; of organic matter such as plant residues, animal faeces and bones and other materials; and of nutrients such as nitrogen (N), phosphorus (P), calcium (Ca), zinc (Zn) and manganese (Mn). It also shows high levels of microorganic activities and other specific characteristics within its particular ecosystem.

It may be added to soils with the intention to improve soil functions and to reduce emissions of biomass (Glaser et al., 2003; Kawa and Oyuela-Caycedo, 2008). Independently, biochar can increase soil fertility (Rondon et al., 2007; Van Zwieten et al., 2007), enhance agricultural productivity (Yamato et al., 2006) and provide protection against some foliar and soil-borne diseases (Elad et al., 2011). Biochar addition to soils has attracted widespread attention as a method to increase soil C sequestration while also reducing atmospheric CO_2 concentrations (Lehmann, 2007; Laird, 2008). Increased soil C sequestration can also improve soil quality because of the vital role that C plays in chemical, biological, and physical soil processes and many interfacial interactions. The study of charcoal use in soil is just beginning, the possibilities are numerous, and the research work yet to be done is enormous (McHenry, 2011).

Plant growth promoting rhizobacteria (PGPR) are one of the viable alternatives to chemical fertilizers (Kloepper et al., 1986; Minaxi and Saxena, 2011). About 2–5% of rhizobacteria, when reintroduced in soil exert a beneficial effect on plant growth. Apart from fixing N_2, many strains of PGPR can affect plant growth directly

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by solubilizing inorganic phosphorus, synthesizing phytohormones and vitamins, inhibiting plant ethylene synthesis, enhancing stress resistance, improving nutrient uptake and mineralizing organic phosphate (Dobbelaere et al., 2003; Lucy et al., 2004; Ogut et al., 2010; Jain et al., 2012). Increased growth and yield of potato, sugar beet, radish and sweet potato have been reported by Farzana et al. (2009) by inoculation of rhizobacteria under green house conditions.

Although PGPR have been extensively documented for their positive impact on plants, but if added with biochar, they not only result in an enhancement of crop yield, but also help in preventing fertilizer run-off, leaching, retaining moisture and helping plants through periods of drought. Most importantly, the combination of PGPR and biochar replenishes exhausted or marginal soils with organic carbon and fosters the growth of soil microbes essential for nutrient absorption. Keeping the above facts in mind a study was planned to assess the growth and yield of French beans (*Phaseolus vulgaris*) in a pot experiment in presence of biochar and a potential PGPR, *Bacillus* sp.

2. Material and methods

2.1. Plant material and PGPR strain

French bean (*P. vulgaris* var. Anupama) seeds were obtained from a local market. The seeds were rinsed with sterile distilled water for 3–4 times, blotted on a sterile filter paper, dried and kept for further use. All the steps were carried out under a laminar flow (LF) clean bench.

The strain *Bacillus* sp. RM2 was originally isolated from rhizosphere soil of *Vigna radiata*. This strain was found genetically stable after a series of sub-culturing, hence chosen for bioinoculation studies. It has been earlier identified by 16S rRNA gene sequence which has been submitted in GenBank with accession number FJ805449 (Minaxi et al., 2012).

2.2. Inoculum preparation

A single colony of the *Bacillus* strain was used to inoculate 50 ml of Luria Bertani (LB) medium and incubated at 30 ± 2° C in an orbital shaker for 24 h at 130 rpm. Jaggery slurry was prepared in d.w, cooled and mixed with bacterial cultures so that bacterial cells stick to the surface of seeds. The seeds were coated with the *Bacillus* culture at a concentration of 10^8 cells ml^{-1}. Seeds coated with medium and jaggery mix only (without inoculum) served as controls. Seeds were kept for drying on a clean, surface sterilized plastic sheet under the LF.

2.3. Experimental design

The experiment was set up in a randomized block design (RBD). It consisted of 6 different treatments, with 4 replicates each. Every pot contained 900 g of unsterilized loamy soil with neutral to slightly alkaline pH. Soil and biochar were thoroughly mixed and sieved (mesh, 2 mm) to remove large particles. The biochar was mixed in soil at the rate of 15 g kg^{-1} of soil. Di-ammonium phosphate (DAP) (0.1 g kg^{-1} soil) and Biozyme (6 g kg^{-1} soil) were added as chemical fertilizer and commercial biofertilizer, respectively. The treatments were as follows: (i) Uninoculated control (UC) (seeds coated with medium and jaggery), (ii) soil + biochar, (iii) soil + biochar + *Bacillus*, (iv) soil + biochar + commercial biofertilizer (Biozyme), (v) soil + *Bacillus*, (vi) soil + DAP.

Ten air-dried seeds of French beans were immediately sown at 2 cm depth (10 seeds pot^{-1}) in 1 kg plastic pots (length, 12.5 cm; upper diameter, 12.0 cm; lower diameter, 8.5 cm) filled with soil, soil with biochar and soil with DAP. For bioinoculation, seeds were coated with jaggery and *Bacillus* suspension; air dried, and then sown in pots containing soil and soil + biochar. The pots were sprinkled with water. The germination rate was calculated 5 days after sowing and seedlings were thinned down to six in each pot. The experiment was set up in an open environment from 3rd April 2012 to 4th June 2012. Plants were watered daily to maintain moisture at field capacity. Three sample plants from each of the four pots were harvested randomly to estimate the parameters such as root and shoot length and biomass, thus a total of 12 replicates were used for measurements within 60 days after sowing.

2.4. Parameters studied

Following parameters were studied during the growth of French bean plants and after their harvest.

2.4.1. Germination rate and shoot and root measurements

Five days after sowing, germinated seeds were counted and the germination rate was calculated by the following formula:

$$\%\,\text{Germination} = \left(\frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}}\right) \times 100$$

Whereas, shoot and root length and fresh biomass (including leaves) were determined after 2 months growth cycle of plants. The plants were harvested taking all the precautions so that roots remained intact.

2.4.2. Yield parameters and Bacillus count

Number of flowers $plant^{-1}$, number of pods $plant^{-1}$, number of seeds $plant^{-1}$ and seed biomass were measured at harvesting time. The bacterial counts were determined at the time of harvesting. The rhizosphere soil, adhering intimately to the roots was separated by gentle tapping and composite samples were prepared. The soil samples were then air dried at room temperature and enumeration of *Bacillus* sp. was done by dilution plate technique on Pikovskaya agar medium to display phosphate solubilizing bacteria. Each plate was replicated 3 times, and incubated for 5 days at 37 ± 2 °C. The soil samples before inoculation of *Bacillus* sp. were also analyzed to calculate their native phosphate solubilizing bacteria which was taken into consideration while estimating the bacterial counts after the bioinoculation.

2.5. Soil and plant tissue analysis

Soil was analyzed for physico-chemical characteristics before the experiment. The method of Allen (1989) was followed to determine the soil pH. For estimation of available phosphorus the samples were first extracted with Olsen's reagent and then available phosphorus was determined according to the method of Olsen et al. (1954), whereas the total phosphorus concentration of soil samples was determined after digestion with perchloric acid (Olsen and Sommers, 1982). Total nitrogen content was estimated by micro-Kjeldahl method (AOAC, 1965). Potassium content of the soil and plant samples was determined by flame photometer.

The oven-dried (at 60 °C for 48 h) shoot samples were processed for the estimation of total nitrogen and phosphorus content. The method consists of digestion of samples followed by distillation process. Kjeltec Auto analyzer 1030 was used for N estimation. Total phosphorus content of samples was estimated by Jackson (1967) method.

2.6. Statistical analysis

Results are expressed as the mean ± standard deviation (SD) of different independent replicates observation as mentioned for

Table 1
Physico-chemical characteristics of soil and biochar.

Sl. No	Sample	pH	Electric conductivity	Organic matter (%)	Total Kjeldahl nitrogen (%)	Total P_2O_5 (%)	Av. P_2O_5 (%)	K (kg ha^{-1})
1	Soil	7.2	28 mS × L/mol	1.48	0.116	0.0126	0.0031	277
2	Biochar	7.8	40 mS × L/mol	12.75	1.03	0.0300	0.0228	340

Table 2
Germination rate (%), root and shoot length/biomass of French beans.

Treatments	% Seed germination	Root length (cm)	Root biomass (g)	Shoot length (cm)	Shoot biomass(g)
Soil	6.75 ± 0.95	10.683 ± 1.01	0.892 ± 0.31	16.716 ± 1.03	1.624 ± 0.68
Soil + *Bacillus*	7.5 ± 0.57*	13.133 ± 1.97*	1.309 ± 0.19*	18.205 ± 2.01*	2.364 ± 0.29*
Soil + Biochar	8.25 ± 0.95*	13.125 ± 2.35*	1.747 ± 0.63*	20.025 ± 1.84*	3.194 ± 0.86*
Soil + Biochar + *Bacillus*	**9.75 ± 0.50***	**14.881 ± 2.16***	**1.846 ± 1.00***	20.183 ± 1.45*	**3.221 ± 0.52***
Soil + Biochar + Biozyme	9.5 ± 0.57*	13.867 ± 1.91*	1.655 ± 0.66*	**20.633 ± 2.66***	3.212 ± 0.89*
Soil + DAP	9.5 ± 0.57*	14.733 ± 2.88*	1.227 ± 0.80*	20.333 ± 2.58*	2.973 ± 0.30*

Values are the mean of replicates ± SD, highest values are shown in bold. Values are significantly analyzed by using *t*-test by SPSS software version 16 at the 5% level of significance ($p \leq 0.05$).
* Significant.

different experiments. The values are analyzed by using *t*-test by SPSS software version 16 at the 5% level of significance ($P \leq 0.05$).

3. Results

The physico-chemical characteristics of soil are presented in Table 1. The pH of biochar was slightly alkaline compared to almost neutral soil pH of 7.2. The percentage of organic matter in biochar was significantly higher than in soil. It was also richer in nutrients like P, N and K.

The isolate used in this study was previously identified as *Bacillus* sp. by Minaxi et al. (2012). It was Gram positive, short rods, non motile with cream pigment and made circular colonies with entire margin. It utilized citrate and carbohydrates such as fructose, lactose, maltose and sorbitol, reduced nitrate and was found positive for catalase and oxidase test, whereas it gave negative results for indole, methyl red and Voges Proskauer tests, hydrolysis of casein, gelatin, urea and starch, H_2S production and utilization of mannitol and sucrose.

Generally, all the treatments showed significantly enhanced growth and yield in comparison to control plants grown in pure soil. It was observed that the addition of biochar influenced the growth of plants positively and inoculation of *Bacillus* sp. further improved the germination rate, growth and yield. Thus, the addition of biochar and *Bacillus* sp. to soil proved to be the best, followed by the combination having biochar and biozyme in most of the cases (Tables 2 and 3; Figs. 1 and 2). The germination rate was highest when soil and biochar were used along with *Bacillus*, followed by the treatments in which biozyme and DAP were added. The root length and root and shoot biomass were significantly higher at $P < 0.05$ i.e. 14.881 cm, 1.846 g and 3.221 g, respectively in the treatment consisting of soil, biochar and *Bacillus* sp. as compared to 10.683 cm, 0.892 g and 1.624 g in uninoculated control. On the contrary, the shoot length was highest in the treatment soil + biochar + Biozyme, however, soil + biochar + *Bacillus* sp. treatment also had significantly higher value than UC. The combination, soil + biochar + *Bacillus* sp. also gave significantly best results for the parameters such as number of pods, number of seeds and seed weight. However, the values for number of flowers were at par when soil was amended either by biochar + *Bacillus* sp. or DAP.

To see whether the inoculated rhizobacterial strain has established itself well in plant rhizosphere or not, cell counts were determined at 2 different time intervals. It is evident from Table 4 that in bioinoculated treatments, the bacterial population increased towards maturation of the crops. The population was maximum when *Bacillus* sp. was inoculated in soil with biochar as compared to when biochar was not mixed in soil. P and N contents in all amended treatments were significantly better than the control (UC) in shoots. Interestingly, P content was maximum in soil + DAP treatment and except for soil + *Bacillus* sp. combination, all other treatments showed significant enhancement. In contrast, all the treatments except for soil and DAP were found to have significant percent N content in plant shoots. Maximum increase however was observed in soil + biochar + *Bacillus*.

One important fact observed during the experiment was that when the pots faced extreme conditions of sunlight, high temperature, etc., the pots containing biochar showed a higher stress tolerance.

4. Discussion

The objective of this study was to assess the biochar and *Bacillus* sp. application on plant growth and yield on French beans. The addition of biochar to soil proved to be beneficial for the growth and yield of the plants. It may be because biochar is known to have many characteristics which are beneficial for the agriculture. Biochar used for the study had a higher content of organic matter and nutrients than the soil used. Addition of biochar to soil has shown definite

Table 3
Enhancement of yield parameters of French bean plants at harvest in pot trial.

Treatments	No. of flowers $plant^{-1}$	No. of pods $plant^{-1}$	Seed no. $plant^{-1}$	Seed biomass (g)
Soil	0.667 ± 0.0135	0.375 ± 0.142	2.28 ± 0.75	0.641 ± 0.66
Soil + *Bacillus*	0.875 ± 0.083*	0.625 ± 0.159*	2.4 ± 0.96*	1.398 ± 0.73*
Soil + Biochar	1 ± 0.137*	0.667 ± 0.00*	2.75 ± 0.96*	1.556 ± 0.80*
Soil + Biochar + *Bacillus*	**1.333 ± 0.215***	**1.083 ± 0.215***	**3.58 ± 0.99***	**3.551 ± 1.15***
Soil + Biochar + Biozyme	1.250 ± 0.021*	0.917 ± 0.096*	2.33 ± 1.07*	2.501 ± 1.05*
Soil + DAP	**1.333 ± 0.136***	0.875 ± 0.083*	2.75 ± 0.86*	1.963 ± 1.45*

Values are the mean of replicates ± SD, highest values are shown in bold. Values are significantly analyzed by using t-test by SPSS software version 16 at the 5% level of significance ($p \leq 0.05$). *Significant.

Fig. 1. Plant growth in different treatments and control.

Fig. 2. Root proliferation in various treatments (a) UC, (b) soil + *Bacillus*, (c) soil + DAP, (d) soil + Biochar, (e) soil + Biochar + Biozyme and (f) soil + Biochar + *Bacillus*.

increases in cation exchange capacity (CEC) and pH (Topoliantz et al., 2002). Verheijen et al. (2009) have reported that biochar application influenced the toxicity, transport and fate of different heavy metals in the soil through improved overall sorption capacity of soils. In a recent study done by Nigussie et al. (2012), it was found that the addition of biochar increased soil pH, electrical conductivity, organic carbon, total nitrogen, available phosphorous, CEC and exchangeable cations of chromium polluted and unpolluted soils.

During the experiment, biochar was added at a rate of 15 g kg^{-1} of soil. The application rate of biochar depends on soil types and crops. Not many studies have been done on its application rate, however, 5–50 tonnes per hectare has been found appropriate. Major et al. (2010) experimented on application rate of biochar to Colombian Savanna Oxisol for 4 years and found that maize grain yield did not significantly increase in the first year, but increases in the 20 t ha^{-1} plots over the control were 28, 30 and 140% for 2nd, 3rd and 4th years, respectively. However, many studies have revealed contradictory results like 165 t of biochar ha^{-1} was added to a poor soil in a pot experiment (Rondon et al., 2007) which resulted in decreased rate of crop yield. Another experiment conducted in USA showed that peanut hull and pine chip biochar applied at 11 and 22 t ha^{-1} reduced corn yields below those obtained in the control plots, under standard fertilizer management (Gaskin et al., 2010). Thus, to avoid the negative impact of biochar

Table 4
Abundances of P solubilizing rhizobacteria and P and N content in rhizosphere of French bean plants and P and N content in shoots at harvest.

Treatments	P solubilizing bacteria counts ($\times 10^6$)	P content (shoot g^{-1})	% N content (shoot g^{-1})
Soil		0.583 ± 0.200	1.14 ± 0.02
Soil + *Bacillus*	1.82 ± 0.06	0.580 ± 0.009^{ns}	1.24 ± **0.15***
Soil + Biochar		0.679 ± **0.021***	1.31 ± **0.07***
Soil + Biochar + *Bacillus*	2.95 ± 0.11	0.689 ± **0.011***	**1.64** ± **0.08***
Soil + Biochar + Biozyme		0.642 ± **0.041***	1.39 ± **0.23***
Soil + DAP		**0.749** ± **0.042***	1.16 ± 0.02^{ns}

Values are the mean of replicates ± SD, highest values are shown in bold. Values are significantly analyzed by using *t*-test by SPSS software version 16 at the 5% level of significance ($p \leq 0.05$). ns, not significant. *Significant.

we added minimal amount of it (approx 3.2 t of biochar hectare^{-1}) and found positive results.

The combined application of biochar with biozyme further improved the overall growth and yield and made this combination second best in many instances. Biozyme is a commercially available biofertilizer which is composed of seaweed extract, soaked neem pellets and bentonite granules (100%). Thus, as a whole it is responsible for increasing the crop yield by promoting the growth of microscopic soil microbes, by stimulating mineral absorption and increasing the strength of root system. It also has the advantage of protecting plants against disease pest.

Many plant growth promoting rhizobacteria have been found to promote the growth of many fruits and vegetables (Manchanda and Singh, 1987; Yasmin et al., 2007). *Bacillus* spp. are commonly found in rhizosphere soil and have been cited in literature to show plant growth enhancing effects (Canbolat et al., 2006; Podile, 1995). Besides, many of the species are also effective for the control of plant diseases caused by soil borne, foliar, and post harvest fungal pathogens (Abeysinghe, 2009; El-hamshary and Khattab, 2008). The isolate selected for the study showed many plant growth promoting activities e.g. the phosphate solubilized by this strain after 24 h of incubation was 175.3 ppm, whereas the highest ACC deaminase activity was found to be 54.14 μM α-ketobutyrate mg^{-1} protein h^{-1} in 24 h growth. It also produced high-level of indole-3-acetic acid (39.47 μg ml^{-1}) in medium in presence of 3 mg ml^{-1} tryptophan and exhibited ammonia production and antifungal activities (Minaxi et al., 2012). All these characteristics show that this strain has potential to be used as bioinoculant. The combination of this strain with biochar had the highest increase in growth and yield of plants compared to all other treatments. The presence of plant nutrients and ash in the biochar, its high surface area and porous nature and capacity to act as a medium for microorganisms have been identified as the main reasons for the increased soil properties and nutrient uptake by plants in biochar treated soils (Nigussie et al., 2012).

French bean seeds coated with *Bacillus* showed a significant increase in germination rate at $P < 0.05$ in comparison to the uninoculated control. The germinating seeds receive most of the nutrients from reserve food material available in endosperm, hence the role of rhizobacteria at this stage is less critical, however, some nutrients and metabolites such as indole-3-acetic acid, gibberelic acid etc. in soil may act as stimulants for seed germination (Tien et al., 1979; Niranjan et al., 2003). Minaxi et al. (2012) have also reported enhanced seed germination by inoculation of *Bacillus* sp. RM2 in cowpea plants.

Root and shoot length and biomass of French beans increased significantly by the amendment of soil with either biochar or *Bacillus* sp., singly or in combination. Many reports have shown that bacteria increased root length in different plants (Glick, 1995; Xie et al., 1996). Such increase in root length may confer advantages to the host system with respect to its health and growth. While extensive development of the adventitious root system increases surface area and consequently the efficiency of nutrient absorption by plants, an increase in root length improves the survival of young seedlings, especially at the initial stage of development (Vasudevan et al., 2002). Also, dual inoculation of phosphate solubilizing bacteria and *Azotobacter chroococcum* had greater positive impact on height of potato plants which could be ascribed to availability of P and N (Faccini et al., 2002).

The higher yield of inoculated plants observed during the experiment could be assigned to increased nutrient availability and uptake by plants. Enhancement of crop yield was observed in pot experiments during the study which is in agreement with the findings of many workers (Cakmakei et al., 2001; Ozturk et al., 2003) who recorded increased yield of wheat by inoculation of *Bacillus* spp. Yield increase obtained in inoculated plants could be attributed to the production of plant growth substances produced by root colonizing bacteria (Kennedy and Tchan, 1992). These might be responsible for well developed root system and enhanced nutrient and water uptake, thereby overall promotion of yield. Our results are in close conformity with those reported by Yadegari et al. (2008) who found a significant increase in pods plant^{-1}, number of seeds pod^{-1}, weight of 100 seeds, weight of seeds plant^{-1} and protein content by co-inoculation of PGPR and *Rhizobium* in beans. Similarly, desirable effects of various inoculations in legumes reported in many experiments (Sindhu et al., 2002; Zaidi et al., 2003) could be assigned to increased nutrient uptake, biological control and other plant growth promoting traits. A few years back, Linu et al. (2009) reported the positive effect of co-inoculation of phosphate solubilizing *Gluconacetobacter* sp. and *Burkholderia* sp. on cowpea which included improved nodulation, higher root and shoot biomass and straw and grain yield. Besides the beneficial role played by rhizobacterial inoculation during the study, biochar may have provided many more nutrients which could have been an added advantage to plants. Liang et al. (2006) and Solomon et al. (2007) also documented higher organic C and total N at the ancient *terra preta* compared to adjacent soils, a positive contribution towards the better growth of plants.

The positive rhizosphere colonization ability of our rhizobacterial strain lies in its being the successful colonizer of the rhizosphere, its capability to increase the seedling growth and its establishment in the rhizosphere of plants giving protection against pathogens, which indirectly resulted in enhanced yield. Hofte et al. (1991) also recorded remarkable root colonization by *Pseudomonas aeruginosa* in various vegetables and cereals. The increase in nutrient contents can also be attributed both to addition of biochar and rhizobacterial inoculation. The increase in the availability of major plant nutrients due to application of biochar was reported by Glaser et al. (2002) and Lehman et al. (2003). In a recent study, the uptake of nitrogen, phosphorous and potassium was found to increase by addition of biochar in *Lactuca sativa* (Nigussie et al., 2012). Similar findings of higher accumulation of P and N in plants in inoculated series have been recorded by Linu et al. (2009). Lehmann and Rondon (2006) and Uzoma et al. (2011) also reported increased nutrient uptake due to addition of biochar in the tropical environment.

5. Conclusions

It can be concluded that both biochar and the bioinoculant, *Bacillus* sp. have the potential to enhance the overall growth of the French beans, hence can be used for sustainable agriculture. The study gives the hope to shift gradually from chemical fertilizers to bio and organic fertilizers. However, subsequent field studies are planned to be carried out in future experiments.

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