Evaluation of biochar-immobilized *Bacillus* spp. as plant growth promoter

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ABSTRACT

Excessive use of chemical fertilizers and pesticides degrades soil fertility and fosters genetic resistance to plant diseases, causing significant agricultural and environmental issues. Beneficial microorganisms like Bacillus spp. enhance plant development, yet direct soil inoculation is ineffective due to bacterial adhesion to soil particles. Carrier materials, such as biochar from agro-industrial waste, offer a solution. This study evaluated biochar-immobilized *Bacillus* spp. on wheat and soybean initial growth. Biochar, derived from grape waste pyrolysis, was inoculated with *Bacillus subtilis* BEIB-18 and *Bacillus megaterium* BEIB-30 and then applied to the soil. Results showed improved root development in both crops, with significant increases in root length, volume, and surface area compared to controls. Biochar treatments outperformed others, indicating its efficacy in enhancing early plant growth. This research demonstrates biochar's potential in promoting seedling development, providing an environmentally friendly and efficient strategy to address agricultural productivity and environmental concerns. The study highlights the importance of early plant growth in achieving high productivity and supports the use of biochar and microorganisms as a sustainable agricultural practice.

Keywords: *Bacillus subtilis*. *Bacillus megaterium.* Pyrolysis. Waste valorization. Sustainable agriculture.

1 INTRODUCTION

Chemical fertilizers and pesticides, when used excessively, can lead to low fertility and genetic resistance to plant diseases, contributing to numerous agricultural and environmental safety problems. (1, 2). The use of beneficial microorganisms, such as *Bacillus* spp., have played a fundamental role in the development and growth of plants (3). Direct inoculation of liquid inoculum into soil may be ineffective, pois the bacteria adhere to soil particles, thus preventing their vertical movement and ability to colonize the root system of plants in the underground soil profile (4, 5). The use of carrier materials is an effective strategy to resolve the limitations of direct application (6).

One of the possibilities for cell immobilization is the use of biochar from agro-industrial waste (7). Studies with biochar based on different organic raw materials have shown distinct effects on plant development related to agronomic efficiency (8, 9). A quick way to evaluate the effects of a given product on plant development are assessments at the initial stage of the crop cycle. Good performance in the first phase of a plant's vegetative cycle is an important indicator of achieving high productivity. (10). In this sense, this study aimed to evaluate the effect of biochar-immobilized with *Bacillus* spp. on the initial development of wheat and soybean crops.

2 MATERIAL & METHODS

Biochar was produced form the pyrolysis of grape wastes at 350 ºC. The company Beifur LTDA supplied the microorganisms *Bacillus subtilis* BEIB-18 (BS) *and Bacillus megaterium* BEIB-30 (BM). For the immobilization of microorganisms, a culture medium was prepared with 7 g/L of soy extract and 13 g/L of sucrose. Then, 10 g of biochar (5% w/v) was added to 200 mL of this medium in 500 mL Erlenmeyer flasks and sterilized for 30 minutes in an autoclave (121 °C; 1.1 atm). The Erlenmeyer flasks were inoculated with two aliquots of *Bacillus subtilis* and *Bacillus megaterium* microorganisms separately. The process of growth and immobilization of microorganisms occurred on a shaking table (Tecnal – TE 420) at a temperature of 30 ºC, 150 rpm, and 24 hours. Subsequently, the biochar-immobilized *Bacillus* spp. was dryed at 45 ºC. Then, the material was transferred to sterile packaging and stored at room temperature.

For the agronomic efficiency test, wheat and soybean crops were used. The experiment had 10 treatments: T1: Soil (control); T2: Soil + NPK; T3: Soil + Biochar; T4: Soil + Biochar + NPK; T5: Soil + BS-biochar; T6: Soil + BM-biochar + NPK; T7: Soil + BS-biochar + NPK; T8: Soil + BM-biochar + NPK; T9: Soil + BS-biochar + BM-biochar; T10: Soil + BS-biochar + BM-biochar + NPK. The design was in randomized block design with five replications and three plants per replication. Plastic pots were used with 250 g of sterile soil (121°C for 30 min in an autoclave) and a dose of 300 kg/hectare of NPK fertilizer was applied and 10% of immobilized *Bacillus*-biochar was added to it. The experiment was maintained in a growth chamber for 15 days at a controlled temperature between 22 and 24 °C and 12 h of light. Irrigation was performed manually as needed. The evaluations were carried out on the roots of the seedlings in order to check whether there was any harmful effect due to the application of the product and also to stimulate its growth. The roots were grouped by the WinRHIZO® software into different classes such as length, surface area, diameter in relation to their total length, volume, very thin roots (RMF, Ø < 0.5 mm), fine roots (RF, Ø of 0. 5 to 2 mm) and

thick roots (RG, Ø > 2 mm). Root growth data in the two tested crops were analyzed using the ANOVA statistical test and subsequently the data were subjected to the Tukey test using the Genes statistical program (GENES - Software for Experimental Statistics in Genetics) (11).

3 RESULTS & DISCUSSION

Table 1. Seedling roots in biochar trials.

	Wheat						
	Comp	**ÁreaSup**	**DiamM**	**VolR**	**RMF**	**RF**	**RG**
T1	9,386 ^{c}	2,641 ^{b}	0,971 ^{a}	0,062 ^{b}	3,154 ^{d}	5,721 ^{b}	0,511 ^{a}
T2	48,976 ^{b}	10,009 ^{a}	0,661 ^{bc}	0,172 ^{a}	24,967 ^{bcd}	22,963 ^{a}	1,046 ^{a}
T3	58,488 ^{ab}	10,643 ^{a}	0,582 ^{bc}	0,155 ^{a}	36,017 ^{bc}	21,462 ^{a}	0,991 ^{a}
T4	57,307 ^{ab}	11,600 ^{a}	0,672 ^{b}	0,194 ^{a}	32,669 ^{bc}	23,285 ^{a}	1,346 ^{a}
T5	62,425 ^{ab}	10,298 ^{a}	0,523 ^{bc}	0,140 ^{ab}	45,191 ^{ab}	16,291 ^{ab}	0,938 ^{a}
T6	58,953 ^{ab}	8,878 ^{a}	0,495 ^{bc}	0,109 ^{ab}	46,328 ^{ab}	12,114 ^{ab}	0,491 ^{a}
T7	54,040 ^{b}	8,532 ^{a}	0,513 ^{bc}	0,109 ^{ab}	39,801 ^{abc}	13,743 ^{ab}	0,495 ^{a}
T8	78,821 ^{a}	11,185 ^{a}	0,456 ^{c}	0,130 ^{ab}	63,005 ^{a}	15,222 ^{ab}	0,590 ^{a}
T9	49,434 ^{b}	9,155 ^{a}	0,595 ^{bc}	0,140 ^{ab}	26,974 ^{bc}	21,580 ^{a}	0,881 ^{a}
T10	40,269 ^{b}	7,864 ^{a}	0,638 ^{bc}	0,128 ^{ab}	21,315 ^{cd}	18,191 ^{a}	0,754 ^{a}
	Soybean						
T1	52,780 ^{ab}	22,787 ^{c}	1,378 ^{b}	0,804 ^{c}	9,047 ^{b}	34,999 ^{ab}	8,734 ^{c}
T2	59,709 ^{ab}	24,880 ^{bc}	1,404 ^{ab}	0,891 ^{bc}	15,051 ^{ab}	34,888 ^{ab}	9,761 ^{bc}
T3	69,757 ^{ab}	31,160 ^{ab}	1,454 ^{ab}	1,149 ^{abc}	16,697 ^{ab}	40,795 ^{a}	12,256 ^{abc}
T4	73,745 ^{a}	34,211 ^{a}	1,503 ^{ab}	1,276 ^{ab}	18,285 ^{a}	40,833 ^{a}	14,614 ^{a}
T5	65,936 ^{ab}	30,996 ^{ab}	1,559 ^{ab}	1,220 ^{ab}	15,918 ^{ab}	36,567 ^{ab}	13,438 ^{ab}
T6	58,191 ^{ab}	26,613 ^{bc}	1,527 ^{ab}	1,020 ^{abc}	16,019 ^{ab}	29,970 ^{ab}	12,198 ^{abc}
T7	52,117 ^{b}	24,955 ^{bc}	1,616 ^{ab}	1,016 ^{abc}	12,694 ^{ab}	28,396 ^{ab}	11,024 ^{abc}
T8	61,897 ^{ab}	30,800 ^{ab}	1,704 ^{ab}	1,308 ^{a}	11,940 ^{ab}	37,235 ^{ab}	12,705 ^{abc}
T9	49,996 ^{b}	26,570 ^{bc}	1,814 ^{a}	1,184 ^{abc}	11,327 ^{ab}	26,737 ^{b}	11,931 ^{abc}
T10	61,517 ^{ab}	28,070 ^{abc}	1,541 ^{ab}	1,096 ^{abc}	13,614 ^{ab}	36,274 ^{ab}	11,588 ^{abc}

Legend: Comp (cm) = length; AreaSup (cm2) = surface area; DiamM (mm) = average diameter; VolR(cm3) = root volume; RMF = very fine roots; RF = fine roots; RG = thick roots; BS = Bacillus subtilis BEIB-18; BM = Bacillus megaterium BEIB-30; T1 = Solo (alone/control); T2 = Soil + NPK; T3 = Soil + biochar; T4 = Soil + biochar + NPK; T5 = Soil + BS-biochar; T6 = Soil + BM-biochar; T7 = Soil + BS-biochar + NPK; T8 = Soil + BM-biochar + NPK; T9 = Soil + BS-biochar + BM-biochar; T10 = Soil + BS-biochar + BM-biochar + NPK. Data analyzed using ANOVA, α=5% and means separated by the Tukey test. Equal letters in the column indicate means without statistical difference.

The root is the part of the plant that plays a crucial role in its development, serving as a stabilizer, absorbing water and nutrients and interacting with soil microorganisms. The results obtained in this work (Table 1) show that there was a difference between the treatments. For the wheat crop, of the seven variables evaluated (Table 1), in six of them (except “thick roots”) the control (T1= soil alone) presented a lower average than the other treatments with a statistical difference ($p<0.05$). For the variable “root length”, the upper means, in order from highest to lowest, were from treatments T8 (Soil + BM-biochar + NPK), T5 (Soil + BS-biochar), T6 (Soil + BM-biochar), T3 (Soil + biochar), T4 (Soil + biochar + NPK). The length of roots in seedlings is an important factor in determining their good development, as the majority of root cells are derived from the meristematic cells of the root apex and then gradually differentiate into various cell types in distinct spatial and functional zones (12). All of the treatments mentioned above that were superior for root length contain biochar, indicating that this compound can help in the first days of plant development.

For “root volume”, treatments T4 (Soil + biochar + NPK), T2 (Soil + NPK) and T3 (Soil + biochar) had higher means and a significant difference from the other treatments, T3 (Soil + biochar) and T4 (Soil + biochar + NPK). For “fine roots” T4 (Soil + biochar + NPK), T2, T9 (Soil + BS-biochar + BM-biochar), T3 (Soil + biochar) and T10 (Soil + BS-biochar + BM-biochar + NPK) showed higher means with statistical difference, except T2 (Soil + NPK), all other treatments were with biochar. This demonstrates that biochar treatments provided better wheat root development. Volume is an important measure, typically of root mass and root tissue density (13). Regarding soybean cultivation (Table 1), of the seven variables evaluated, for five of them (surface area, average diameter, volume of roots, very thin roots and thick roots) the control (T1 = soil alone) presented the lowest mean with statistical difference in relation to other treatments. Thick roots are considered to have an anchoring function, their shape, size and architecture are important in their essential functionality and the spatial anatomy of the roots contributes to the growth and development of plants (14,15,16). While fine roots play a vital role in the flow of energy and material in plants, their growth is an important strategy for acquiring nutrients (17).

T4 (soil + biochar + NPK) presented a statistically higher average for five variables (root length, surface area, very fine roots, thin roots and thick roots). Followed by T9 (Soil + BM-biochar + BS-biochar) for average diameter and T8 (Soil + BM-biochar + NPK) for root volume, these were the treatments with the highest averages, that is, all with biochar in their composition, demonstrating that biochar helped in the development of soybean roots. Microorganism treatments can alter the dynamics of plant roots. The combined use of biochar/microorganism/NPK demonstrated diameters of 38.53% for B. subtilis and 24.03% for B. megaterium, larger compared to the control (Table 2) Microorganisms help the roots to increase nutrient supply and water (18), thus playing an important role in the growth of seedlings in the first fifteen days of their development. The results obtained in this study show the potential of using biochar and microorganisms in crop development. With the need to increase the productivity of crops of agronomic importance, identifying the good initial development of seedlings is a fundamental factor in accelerating the process of producing the food necessary for human survival (19).

4 CONCLUSION

The seedling test in this study showed beneficial factors in a short period, which can influence the productivity of a crop. Regardless of the biochar treatment, there was no harmful effect on the seed and statistically. Biochar showed a positive effect on the development of seedling roots. The positive statistical difference in relation to the control shows the potential of biochar in promoting seedling growth in both crops. In this sense, this research provides an environmentally friendly and efficient strategy to contribute for agricultural and environmental problems through the combination of biochar and microorganisms.

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