Australian Journal of Crop Science
AJCS

AJCS 13(07):1183-1187 (2019) ISSN:1835-2707
doi: 10.21475/ajcs.19.13.07.p1779

The synergistic effect of sewage sludge biochar and *Trichoderma harzianum* on soybean yield

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Abstract

The use of sewage sludge to produce biochar is one of the best alternatives for the final destination of this material, allowing for reuse of nutrients and reducing the dependence on mineral fertilizers. Sewage sludge biochar (SSB) stands out as an enhancer of the physical, chemical and biological properties of the soil. The use of beneficial microorganisms such as *Trichoderma* spp. in combination with biochar may have a synergistic effect on the development of different plants and needs to be better studied. The SSB was produced from sewage sludge biomass (SS) obtained from the sewage treatment plant (STP) of the Federal District Environmental Sanitation Company (CAESB), Brasilia, DF, Brazil. The SSB was produced in an electric tubular furnace at 500 °C and showed the following characteristics: carbon (19%), nitrogen (2.3%), hydrogen (1.7%), specific surface area (52.5 m^2 g^{-1}), pore volume (0.053 ml g^{-1}). An experiment was carried out in a greenhouse to evaluate the synergistic effect of SSB application (0.5% w/w) in combination with *T. harzianum* (TH) on soybean development. The treatments adopted were: (1) control – autoclaved soil, (2) TH, (3) SSB and (4) SSB + TH. The SSB was applied 15 days before soybean planting and the TH was applied to the soil two times, once at eight days before planting and the other at the time of planting. The SSB resulted in a 200% increase in the number of pods when compared to the exclusive application of *T. harzianum*. Application of SSB with *T. harzianum* increased germination by 20%, as well as a 70% increase in fresh and dry soybean mass in relation to the control. The agronomic indices evaluated in this study demonstrated that the use of SSB in conjunction with *T. harzianum* presents a synergistic effect, allowing for better development of the soybean plants.

Keywords: *Glycine max,* pyrolysis, biological agent, productivity, soil amendment.
Abbreviations: SS_sewage sludge; SSB_sewage sludge biochar; BCH_biochar; TH_*Trichoderma harzianum;* STP_sewage treatment plant; PSG_percentage of seed germination

Introduction

Sewage sludge (SS) is an organic waste that has great potential for use in agriculture. When applied to the soil, SS increases the organic matter (OM) and nutrient content, with emphasis on nitrogen (N) and phosphorus (P). In addition to increasing nutrient availability, SS can improve soil physical and biological properties (Cézar et al., 2012).

Despite the recognized role of SS in soil fertilization, the occurrence of pathogenic microorganisms restricts its use to few locations. Thermal treatment by pyrolysis promotes the elimination of pathogens and dangerous organic compounds (Caballero et al., 1997), and the main product of this process is biochar (BCH).

Studies have shown that BCH can reduce nutrient losses through leaching (Yuan et al., 2016), provide available nutrients to plants (Furtado et al., 2016; Faria et al. 2018), alkalinize acidic soils (Deenik and Cooney 2016), in addition to innumerable others benefits, favoring improved plant development (Han et al., 2016).

Trichoderma is among the most studied and used biocontrol agents of plant diseases in the world. These free-living and highly interacting microorganisms in the soil, root surfaces and plant tissues (Rajendiran et al., 2010) have antagonistic activity against different phytopathogenic fungi. The inoculation of soybean seeds with *T. harzianum* has resulted in increased plant growth even in soils under saline conditions (Khomari et al., 2018).

In soil, *Trichoderma* is favored by the presence of organic matter. The use of *Trichoderma* in combination with organic waste increased the available P levels, and also favored higher carbon fixation in the soil (Shukla et al., 2017). Muter et al. (2017) observed that the application of BCH together with *Trichoderma* resulted in increased germination of corn seeds, as well as taller plants.

Considering that BCH not only provides plant with nutrients but also interferes with the soil microbial, there is still a lack of information regarding the effects of BCH on the action of beneficial soil organisms. Graber et al. (2010) observed that in soils receiving BCH there was an increase in the population of *Trichoderma* spp. in relation to the control soil (without BCH). Hu et al. (2014) also reported changes in composition of the fungi community after application of BCH to the soil, revealing a proportion of *Trichoderma* 14.5% higher than that observed in soil that did not receive BCH.

Table 1. Physicochemical characteristics of the sewage sludge and biochar.

Characteristics	Sewage sludge	Biochar
TC (%)	21.0	19.0
TN (%)	3.0	2.3
NO_3^- (mg kg^{-1})	-	5.8
NH_4^+ (mg kg^{-1})	-	169.3
pH ($CaCl_2$)	4.8	6.5
P (g kg^{-1})	35.7	61.3
K^+ (g kg^{-1})	0.8	1.2
Ca^{+2} (g kg^{-1})	6.6	7.8
Mg^{+2} (g kg^{-})	0.86	1.7
S (g kg^{-1})	-	7.4
B (mg kg^{-1})	-	12.0
Cu (mg kg^{-1})	-	1.1
Fe (mg kg^{-1})	-	541
Mn (mg kg^{-1})	-	1.2
Zn (mg kg^{-1})	-	6.2
FA	-	4.3
HA	-	1.1
Humin	-	95.6
PV (mL g^{-1})	-	0.05
SSA (m^2 g^{-1})	-	52.5
Thermotolerant coliforms [(a)]	-	<1
Helminths [(b)]	-	0
Moisture content (g g^{-1})	0.20	-
Volatile matter (g g^{-1})	0.45	-
Ash (%)	54	-

TC, total carbon; TN, total nitrogen; FA, fulvic acid; HA, humic acid; PV, pore volume; SSA, specific surface area; (a) (MPN g^{-1} of dry matter); (b) (viable eggs g^{-1} of dry matter).

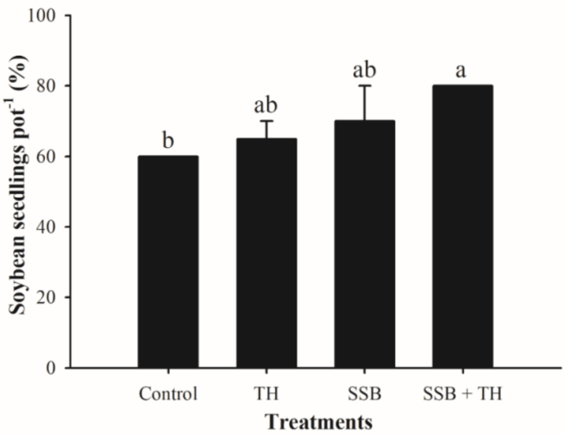

Fig 1. Percentage of soybean seedlings (*G. max*) per pot in the different treatments (SSB -biochar 500 °C, TH - *T. harzianum*). The same letters indicate that there is no significant difference [Duncan's test (p<0.05)]. The bars indicate standard deviation of the mean.

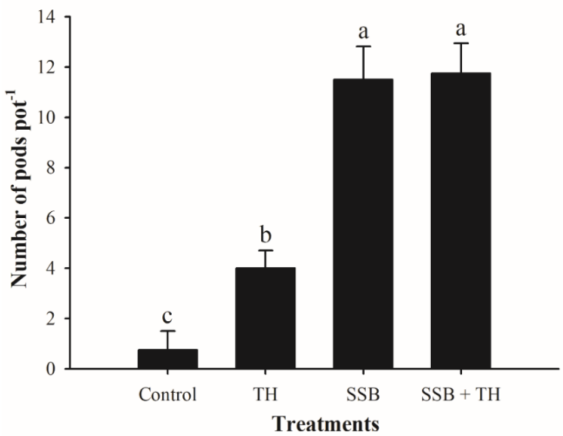

Fig 2. Number of soybean pods (*G. max*) per pot in different treatments. (SSB - biochar 500 ºC, TH - *T. harzianum*). The same letters indicate that there is no significant difference [Duncan's test (p<0.05)]. Bars indicate the standard deviation of the mean.

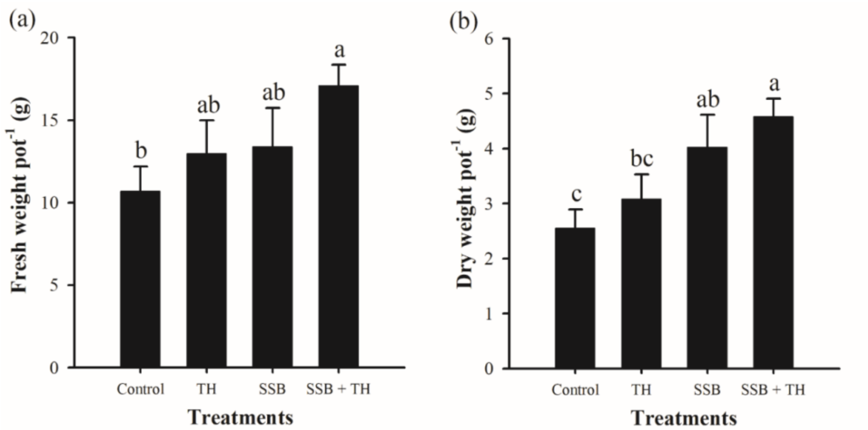

Fig 3. Fresh (a) and dry mass (b) production of soybean plants (SSB-biochar 500 ºC, TH - T. harzianum). The same letters indicate that there is no significant difference [Duncan's test (p <0.05)]. Bars indicate the standard deviation of the mean.

Additionally, studies suggest that induction or limitation of plant growth occurs by the direct effect of *T. harzianum* on root development, in combination with indirect mechanisms such as solubilization of minerals, including solubilization via acidification, redox, chelation and hydrolysis (Li et al., 2015). In this sense, the combined use of *T. harzianum* must be better understood in order to develop sustainable technology for agricultural use. The objective of this study was to evaluate, using different agronomic indices, the effects of SSB and *T. harzianum*, applied alone or in combination, on soybean development.

Results and Discussion

Effect of biochar and T. harzianum on soybean seedlings

The exclusive application of SSB or *T. harzianum* to the soil did not increase the germination of soybean seeds compared to the control (p<0.05). However, the combined use of SSB + *T. harzianum* resulted in a 20% increase in soybean germination in relation to the control (Fig. 1). These results indicate a synergistic effect between SSB and *T. harzianum* that may be the result of compositional changes in the soil microbial community, resulting in an increase in the population of *Trichoderma* spp. (Graber et al., 2010, Hu et al., 2014). This increase in the *Trichoderma* population may have favored a greater amount of nutrients in the soil, for example, a possible increase in available P contents (Shukla et al., 2017).

Muter et al. (2017) observed that the application of pelletized biochar from wheat straw together with *T. viride* resulted in higher germination of maize seeds, as well as increased growth (height of plants) in relation to the treatments with biochar and *T. viride* applied alone. The ability of biochar to protect *Trichoderma* favored its survival in the soil and the direct role of microorganisms on plant growth is indicated as possible explanations for the beneficial effects obtained. Furthermore, there is also the possibility that *Trichoderma* makes nutrients present in the biochar available to the soil.

Effect of biochar and T. harzianum on number of soybean pods

Both SSB and *T. harzianum* increased the number of soybean pods relative to the control. However, SSB stood out and promoted a number of pods 200% greater than the application of *T. harzianum* alone (p<0.05). The best results are associated with the use of SSB, applied alone or in conjunction with *T. harzianum*, demonstrating its effective capacity to increase soybean production (Fig. 2).

The effects of BCH addition on crop productivity have previously been demonstrated in several studies. These studies indicate direct and indirect effects of the action of BCH on crop yield. Kraska et al. (2016) attributed the nutritional effects of BCH on rye productivity to increased availability of nutrients, including P, Mg and K.

Similarly, Ibrahim et al. (2017) justified the higher bean yield to improved soil fertility indicators promoted by the SSB, with a 21% increase in biomass of the aerial portion and 27% in the number of fruits. Graber et al. (2014) highlighted the possible effect of BCH on the rhizospheric system complex, indicated by the interaction between root/soil/pathogen due to a series of physical and chemical properties presented by BCH, including: nutrient content, water retention capacity, redox activity, adsorption capacity, pH and content of toxic and hormonal compounds, potentially influencing plant development.

Effect of biochar and T. harzianum on the fresh and dry mass production of soybean plants

The fresh mass and dry mass production of soybean plants showed a strong and positive interaction of SSB with *T. harzianum* (Fig. 3). This synergistic effect guaranteed an increase in soybean plant mass of approximately 70% in relation to that observed in the control.

Different studies associate the use of BCH with the increase of nutrients and carbon in the soil, resulting in greater plant development (Abrishamkesh et al., 2015; Faria et al., 2018). Scheifele et al. (2017) verified not only the nutritional effects provided by the biochar when applied to the soil, but also a positive influence on the formation of nodules responsible for biological nitrogen fixation and an increase of dry mass in soybean plants conducted in certain soils with application of BCH from corn crop remains.

The results of individual application of SSB or *T. harzianum* did not express a significant increase in fresh plant mass, confirming that combined application of the two products is highly favorable for soybean development.

Seed treatment with *T. harzianum* resulted in an increase in bean plant growth, resulting in greater leaf area, root area and higher number of secondary roots (Pereira et al., 2014). Similarly, *Trichoderma viride* applied to the soil altered the biodynamics of rye plants (*Secale cereale* L.), increasing the number of leaves in relation to the control (Vecstaudza et al., 2018). The authors observed that use of wood biochar resulted in better survival of *Trichoderma* spp. during the 35 experimental days, and consequently, better development of the plants.

Materials and Methods

Acquisition and characterization of the biochar

The BCH was produced from SS biomass from the sewage treatment plant (STP) of the environmental sanitation company of the Federal District, Brazil. This STP uses a tertiary treatment system, in which the SS is treated in an upflow anaerobic reactor (UAR), biological reactor and clarifier. The SS used was dried and its characteristics are described in Table 1.

The SSB was produced in an electric tubular furnace (Linn Elektro Therm) at 500 °C, with temperature rise rate equal to 11°C min^{-1} and residence time of 30 minutes. The SS samples were placed in a metal vessel adapted to the internal space of the furnace with a gas and bio-oil exit system, and a mechanism that restricts the flow of oxygen. The physicochemical characteristics of the SSB are presented in Table 1.

The SSB surface areas and pore volumes were determined by N_2 adsorption isotherms at -196.2 °C in a surface area analyzer, NOVA 2200. Total C, N and H contents in the SSB were determined using a CHN Elemental analyzer. The pH was determined in a $CaCl_2$ 0.01 M solution, using a 1:5 (w/v) biochar:solution ratio suspension. Electrical conductivity (EC) was measured in a 1:10 (g mL^{-1}) ratio using a conductivity meter. Dried and ground samples were subjected to acid digestion according to USEPA3050B. Macro and micronutrient contents were determined after nitric-perchloric acid digestion. Nitrate and ammonium were determined by the Kjeldhal method. Humic substances were extracted with a NaOH 0.1 M solution. Total coliforms were determined according to American Public Health Association - APHA and the number of helminth eggs was obtained according to Ayres (1991).

Implantation and conduction of the experiment in a greenhouse

The study was conducted in a greenhouse and the development of soybean (*Glycine max*) cultivated in pots was evaluated. For the assay, soybean seeds of cultivar P98C81 were used. Sowing of the soybeans occurred in 4 L pots with autoclaved soil, using 5 seeds per pot. The experiment was conducted in a randomized complete block design with 4 replicates. The following treatments were studied: 1) plant (control), 2) plant + *T. harzianum* (TH), 3) plant + SSB, 4) plant + *T. harzianum* + SSB (SSB + TH). The amount of SSB applied to the soil was 20 g per pot, which represents 0.5% of the total soil weight used in each pot. The SSB was applied 15 days before soybean planting, and was completely incorporated into the soil.

The fungus *T. harzianum* was obtained from the commercial product Tricodermil®, strain 1306, cultivated in PDA (potato-dextrose-agar) culture medium. *T. harzianum* was applied to the soil two times, once at eight days before planting and the other at the time of planting, establishing an application of 40 mL per pot of the conidial suspension at a concentration of $1x10^6$ conidia mL^{-1}, using distilled-sterilized water.

Evaluations of plant development

The evaluations began on the eighth day after sowing the cultivars, adopting a system for observations every 48 hours. The variables evaluated were percentage of seed germination (PSG), number of pods, and fresh mass and dry mass of the plants in each pot. The PSG was established 13 days after sowing, obtained from the number of emergent seedlings per pot. The number of pods was determined at the end of the experiment, counting the number of fully formed pods per pot. At 87 days after germination, the soybean plants were collected and weighed to obtain the fresh mass, considering both the aerial part and roots. Subsequently, they were dried in a forced circulation oven, at 60 ºC for 48 hours. After this period, it was verified that the mass (g) of the plants had stabilized, and consequently a new evaluation was performed, representing the dry mass of the aerial part and roots.

Statistical analysis

The analyses were performed using the IBM SPSS Statistics program, version 23.0 (IBM, 2015). Data was submitted to analysis of variance and means were compared by the Duncan test ($p<0.05$).

Conclusion

The agronomic indices (germination, number of pods, fresh and dry mass) evaluated in this study demonstrated that the use of SSB together with *T. harzianum* caused better development of soybean plants. The results reported in this study indicate synergism between SSB and *T. harzianum*, and that the combined application of these two products is a biological technology alternative that can be used to sustainably increase crop productivity.

Acknowledgements

L.E.B. Blum and C.C. Figueiredo thank CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for the Research Productivity Fellowship.

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