

## **Original Research Article**

# **Compounded biological soil conditioner for soybean crop production**

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### **ABSTRACT**

**Aim:** To evaluate the agronomic efficiency of Bacillus-based soil conditioner on soybean crop development and productivity in different cropping areas.

**Study design:** Randomized complete block design with six treatments and eight replications.

**Place and Duration of Study:** Municipalities of Uberlândia, Paracatú, Araxá and Guarda Mor, all in Minas Gerais State, Brazil, during the 2019/20 season.

**Methodology:** The treatments consisted of untreated control, 300 kg ha<sup>-1</sup> of mineral fertilizer, 2, 2.5, 3, 3.5 L ton<sup>-1</sup> of Bacillus-based soil conditioner applied to mineral fertilizer. Leaf and grain P and K concentration, soybean yield components (grains per pod, pods per plant, weight of a thousand grains) and grain yield were evaluated. Data were subject to ANOVA at  $P = 0.10$ . Treatments means were separated using the Duncan test at a 0.10 level of significance.

**Results:** In sandy clay texture soil (Uberlândia), 2 L ton<sup>-1</sup> of soil conditioner was more efficient than mineral fertilizer, increasing the soybean P leaf concentration; in a clayey soil (Guarda Mor), the most responsive dose of soil conditioner was 3.5 L ton<sup>-1</sup>. 3 L ton<sup>-1</sup> of soil conditioner was more efficient than mineral fertilizer in increasing grain yield by increasing soybean pods per plant and soybean yield (5,600 kg ha<sup>-1</sup>) in Araxá. 3.5 L ton<sup>-1</sup> of soil conditioner generated a higher grain yield in Paracatú (3,860 kg ha<sup>-1</sup>) and Guarda Mor (4,836 kg ha<sup>-1</sup>).

**Conclusion:** This study revealed that using Bacillus-based soil conditioner on soybean crops is a valuable strategy to increase soybean yield and sustainability of agricultural activity.

*Keywords: Bacillus, Glycine max, Soybean yield, Mineral fertilizer.*

### **1. INTRODUCTION**

Soybean (*Glycine max* L.) is a relevant economic, social, and strategic crop worldwide. Brazil is the current highest soybean producer, and the Brazilian soybean crop production was over 122 million tons of grain in the 2021/22 harvest, with an average grain yield of 3,016 kg ha<sup>-1</sup> [1] especially because of the world crisis in the supply of mineral fertilizers due to the pandemic and problems in Europe. This mark is mainly due to advances in scientific research and technologies that increase yield for different regions, such as mineral fertilizers [2] [3]. Nutritional balance is a key factor in improving seed quality and increasing crop productivity [4] [5].

An intensive soybean cultivation system is required to maintain high grain productivity, and this cultivation system requires high amounts of nutrients from fertilizers, representing high economic and environmental costs. Agricultural activity sustainability depends on reductions in inputs used, such as mineral fertilization, and improvements in nutrient use efficiency [6] [7]. Thus, integrating biological fertilization techniques and cultural practices emerges as

soybean crop nutritional management alternatives. In this sense, soybean producers are always looking for other options to enhance productivity, including biological soil conditioners [8] [9] [10] [11].

Microbiology, biochemistry, and plant physiology have identified new compounds and applied them to plants and soil to make them more efficient and productive. Microorganisms are essential and dynamics in the cycle of nutrients in all ecosystems and actively participate in many important processes. Such processes include soil structuring, biological nitrogen fixation, solubilization of nutrients for plants, nutrient cycling, reductions of pathogens, insects and weeds, degradation of persistent agronomic compounds, improved mycorrhizal associations, and many other soil and environmental attributes that directly and indirectly affect plant responses and yield [12].

The PGPR (plant growth-promoting rhizobacteria) can contribute to maintaining soil fertility and improving plant growth [13] [14]. These bacteria that live naturally on the surface or in association with the plant's root system have shown satisfactory results, positively affecting the growth of roots and shoots of plants [15] and disease resistance [16] [17]. PGPR also exerts positive effects on plant nutrition and development by improving the absorption of phosphorus (P) (phosphate solubilization), synthesis of phytohormones (e.g., acetic indole acid), biotic nitrogen fixation, and the control of the deleterious effects of plant pathogens [18] [19] [20].

The *Bacillus* sp. genus can act as a plant growth promoter and is considered a PGRP [21] [22] [23]. This genus is one of the most important rhizobacteria to increase plant growth and positively influence crop germination, development, and yield to produce growth-promoting substances [24] [25]. Additionally, these bacteria present favorable characteristics to commercial inoculants, such as endospore production, safer handling, easy application, and the possibility to mix with other products [21]. Besides presenting the ability to colonize the rhizosphere and promote plant growth, such bacteria are beneficial for nutrient recycling. They can act as biofertilizers which increase crop productivity and improve plant performance during stressful periods [26] [27] [28] [29] [30] [31] [32].

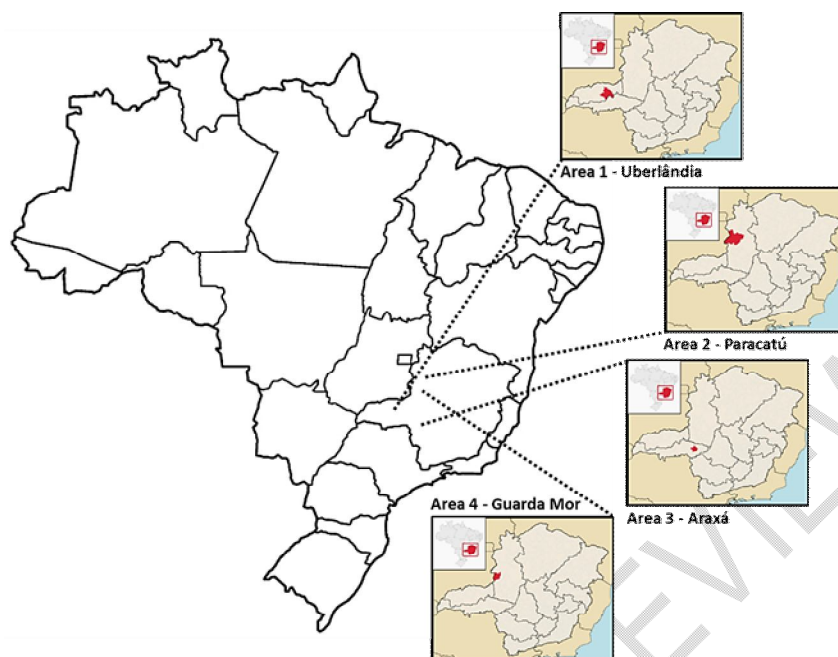
Studies conducted by Jain et al. [33] showed that *Bacillus* sp. isolates could increase, in soybean, the fresh weight of shoots and roots, besides increasing the number of lateral roots. Studies aimed at evaluating the use of organic products are important since using *Bacillus* sp. as growth promoters, and biological control agents can improve the potential of crop production and the sustainability of soil activity [34].

Therefore, this study aimed to evaluate the agronomic efficiency of *Bacillus* soil conditioner based on the soil activity, development, and soybean productivity in different crop-producing areas.

## 2. MATERIAL AND METHODS

### 2.1 Experimental Design

The experiments were implemented in different edaphoclimatic areas in the municipalities of Uberlândia (area 1), Paracatú (area 2), Araxá (area 3), and Guarda Mor (area 4), in Minas Gerais state, Brazil (Fig. 1) during the 2019/20 growing season. The experimental design was set as randomized complete blocks with six treatments (control; 300 kg ha<sup>-1</sup> of mineral fertilizer (0-20-20); 300 kg ha<sup>-1</sup> of mineral fertilizer treated with the soil conditioner at 2, 2.5, 3, and 3.5 L ton<sup>-1</sup> of fertilizer), and eight replications.



**Fig. 1. Map of the location of the soybean planting areas, which are located in Minas Gerais State, Brazil.**

The experimental plots were 3 m wide by 10 m in length each (30 m<sup>2</sup>). The evaluations occurred only in the central two meters of each plot discarding 0.5 meters on each side at the beginning and the end of the plot (18 m<sup>2</sup> useful plot). The treatments were applied only once at planting using a six-line planter (0.5 m spacing between sowing lines).

## 2.2 Experimental Areas

The experiment in Area 1 was implemented at the Físio-Plant Research and Development Experimental Station (road BR 050, km 83.5) in the municipality of Uberlândia, at 18°99'69.5" latitude S and 48°18'86.1" longitude W, at 842 meters altitude. The experiment went from December 2019 to April 2020. The soil is classified as a red Oxisol of sandy clay texture [35]. The climate was classified as 'Aw' according to Köppen's classification.

The soybean cultivar (KWS RK 6719 IPRO) was sown in the conventional tillage and had a population density of 15 plants per linear meter. Seedling emergence occurred seven days after sowing. The soil chemical characteristics (0-0.2 m) were as follows: 56% of sand, 4% of silt, and 40% of clay; pH (H<sub>2</sub>O) 5.8; 23.8 g kg<sup>-1</sup> of organic matter; 39 mg dm<sup>-3</sup> of K; 1.06 mg dm<sup>-3</sup> of P; 1.4 cmolc dm<sup>-3</sup> of Ca, and 0.6 mg dm<sup>-3</sup> Mg.

The experiment in Area 2 was implemented at Tia Dora Farm in the municipality of Paracatú, at 17°09'51.2" latitude S and 46°24'02.7" longitude W, at 696 meters altitude. The experiment went from December 2019 to May 2020. The soil is classified as a yellow-red Oxisol of clayey texture [35]. The climate was classified as 'Aw' according to Köppen's classification.

The soybean cultivar sown was "CZ48B32 IPRO" which was sown in the conventional planting system (tillage) and had a population density of 15 plants per linear meter. Seedling emergence occurred seven days after sowing. The soil chemical characteristics (0-0.2 m)

were as follows: 60% of clay, 27% of silt, and 13% of clay; pH (H<sub>2</sub>O) 6.4; 14 g kg<sup>-1</sup> of organic matter; 21 mg dm<sup>-3</sup> of K; 11 mg dm<sup>-3</sup> of P; 3.9 cmolc dm<sup>-3</sup> of Ca, and 0.9 mg dm<sup>-3</sup> Mg.

The experiment in Area 3 was implemented at Tia Dora Farm in the municipality of Araxá, at 19°56'89.3" latitude S and 46°98'82.5" longitude W, at 910 meters altitude. The experiment went from December 2019 to April 2020. The soil is classified as a yellow-red Oxisol of sandy texture [35]. The climate was classified as 'Aw' according to Köppen's classification.

The soybean cultivar sown was "8473 RSF" which was sown in the conventional planting system (tillage) and had a population density of 18 plants per linear meter. Seedling emergence occurred seven days after sowing. The soil chemical characteristics (0-0.2 m) were as follows: 24% of clay, 10% of silt, and 66% of clay; pH (H<sub>2</sub>O) 5.8; 156 mg dm<sup>-3</sup> of K; 5.5 mg dm<sup>-3</sup> of P; 4.2 cmolc dm<sup>-3</sup> of Ca, and 1.4 mg dm<sup>-3</sup> Mg.

The experiment was implemented at São Severino Farm (road MG 188) in the municipality of Guarda Mor, at 17°39'29.0" latitude S and 47°03'18.8" longitude W, at 598 meters altitude. The experiment went from December 2019 to May 2020. The soil is classified as a red Ultisol of clayey texture (Santos et al., 2013). The climate was classified as 'Aw' according to Köppen's classification.

The soybean cultivar sown was "8473 RSF" which was sown in the conventional planting system (tillage) and had a population density of 15 plants per linear meter. Seedling emergence occurred seven days after sowing. The soil chemical characteristics (0-0.2 m) were as follows: 43% of clay, 15% of silt, and 42% of clay; pH (H<sub>2</sub>O) 5.8; 1.9 mg dm<sup>-3</sup> of K; 94 mg dm<sup>-3</sup> of P; 3.9 cmolc dm<sup>-3</sup> of Ca, and 1.7 mg dm<sup>-3</sup> Mg.

### 2.3 Crop Fertilization and Biological Soil Conditioner

The recommendations for correctives (lime) and fertilizers for Minas Gerais state (Ribeiro, 1999) were defined according to each area's soil type, chemical and physical characteristics. Fertilizer application at planting for all treatments was performed with 300 kg ha<sup>-1</sup> of formulated 0-20-20 (N, P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O), except for the control treatment. The biological soil conditioner was applied in a mixture with the granular fertilizer at rates of 2, 2.5, 3, and 3.5 L ton<sup>-1</sup> of fertilizer.

The biological soil conditioner used in this study is a liquid oil-water emulsion containing four different non-pathogenic microorganisms composed of Bacillus species with a total concentration of 3.01x10<sup>8</sup> propagules per milliliter, in 99% oil-based culture medium. The resulting solution presents dark brown color to black, with 6 to 7 pH and a density of 0.93 ± 0.2 g mL<sup>-1</sup> (20 °C).

The climatic data during the experimental period were obtained in an automatic station located in each experimental area (Table 1).

**Table 1. Dates and climatic conditions at the treatment's applications in each experimental area**

Area	Date	T (°C)	RH (%)	WS (km h <sup>-1</sup> )	C (%)	t <sub>0</sub> (h)	t <sub>final</sub> (h)
Uberlândia	12/10/2019	27.5	71	0.5	100	16:00	18:30
Paracatú	12/14/2019	25.0	70	0.3	100	09:00	12:00
Araxá	12/18/2019	26.5	70	0.2	100	13:00	17:00
Guarda Mor	12/27/2019	26.0	73	0.4	100	08:00	11:00

*T*: temperature; *RH*: relative humidity; *WS* ( $\text{km h}^{-1}$ ): wind speed; *C*: cloudiness;  $t_0$ : initial time of measurement;  $t_{\text{final}}$ : final time of measurement.

In addition, data on temperature, precipitation, and relative humidity were recorded during the experimental period (Table 2).

**Table 2. Meteorological data recorded during the experimental period in each experimental area**

Area	Air temperature (°C)			Precipitation (mm)			Air relative humidity (%)		
	Min.	Max.	Average	Min.	Max.	Average	Min.	Max.	Average
Uberlândia	20.15	28.40	24.82	0.00	69.60	7.66	45.00	89.50	66.99
Paracatú	21.00	30.00	24.56	0.00	111.94	5.86	52.00	98.00	79.67
Araxá	19.70	26.90	23.68	0.00	104.00	9.53	57.00	83.00	69.87
Guarda Mor	18.60	26.55	23.17	0.00	99.80	7.22	46.50	88.50	74.45

*Min.*: minimal average temperature observed in one day during the experimental period; *Max.*: maximum average temperature observed in one day during the experimental period; *Average*: average of all temperatures recorded during the experimental period.

## 2.4 Soybean Evaluations

After the treatment's application P and potassium (K) contents were evaluated in leaves and grains, and yield components: number of grains per pod (GP), number of pods per plant (NP), weight of a thousand grains (WTG) and yield ( $\text{kg ha}^{-1}$  and  $\text{bags ha}^{-1}$  – each bag = 60 kg). The P and K contents in the leaves were determined according to the methodology proposed by [37]. For leaf analysis, ten trifoliolate leaves (newly mature, without petiole, corresponding to the third or fourth leaf from the apex of the main stem) were collected per plot when the plants were in stage 60 of the BBCH scale. From the harvested grains, 100 g were sampled, dried in an oven at 60 °C, ground, and submitted to chemical analysis to evaluate P and K contents [38].

The WTG was determined by accounting for 1,000 seeds per plot that were weighed on a digital scale. The number of pods with one, two, three, and four grains and the total number of pods per plant were measured by counting the variables in 10 representative plants per plot. Crop yield was estimated at the end of stage 99 of the BBCH scale. The grains harvested in the 18 m<sup>2</sup> useful plot were weighed and adjusted to 13% moisture.

When necessary, the data of the evaluations were transformed and submitted to analysis of variance. The averages were compared by Duncan's test of averages ( $p < 0.10$ ) using the software SASM-Agri [39].

## 3. RESULTS AND DISCUSSION

The P and K concentration in soybean plant leaves in the four experiments are presented in Table 3. In none of the areas showed differences between treatments for K leaf concentration were identified. In Area 1 and Area 2, the K grain concentration did not differ ( $p > 0.10$ ). In Area 1, a significant increase in P ( $4.25 \text{ g kg}^{-1}$ ) leaf concentration was identified for plants that received soil conditioner at  $2 \text{ L ton}^{-1}$ . Regarding the P grain concentration, the soil conditioner doses of 2.5 and  $3.5 \text{ L ton}^{-1}$  were more efficient than the other treatments. No difference was observed for K leaf and grain concentration (Table 3).

**Table 3. Phosphorus (P) and potassium (K) content in leaves and grain as a function of treatments applied to soybean in each experimental area**

Treatment	Dose (L ton <sup>-1</sup> )	Leaf Concentration (g kg <sup>-1</sup> )		Grain Concentration (g kg <sup>-1</sup> )	
		P	K	P	K
----- Uberlândia -----					
1. Control	-	3.70 bc*	17.00 a	4,38 b	17,75 a
2. Mineral fertilizer	-	3.93 b	16.50 a	4,55 b	17,75 a
BSC	2	4.25 a	16.50 a	4,58 b	17,50 a
BSC	2.5	3.70 bc	17.00 a	4,78 a	18,12 a
BSC	3	3.70 bc	16.63 a	4,55 b	17,75 a
BSC	3.5	3.58 c	16.38 a	4,78 a	17,62 a
CV (%)		9.47	6.86	4,86	2,53
Average		3.80	16.66	4,60	17,75
----- Paracatu -----					
1. Control	-	1.65 c	19.25 a	3,95 b	14,58 a
2. Mineral Fertilizer	-	2.17 b	19.38 a	5,00 a	15,68 a
BSC	2	2.72 a	21.25 a	4,48 a	15,15 a
BSC	2.5	2.80 a	21.25 a	4,50 a	15,68 a
BSC	3	2.65 a	20.75 a	4,63 a	15,60 a
BSC	3.5	2.77 a	20.75 a	4,65 a	15,35 a
CV (%)		12.37	10.47	10,61	7,44
Average		2.46	20.43	4,53	15,33
----- Araxá -----					
1. Control	-	4.75 b	25.63 a	3,75 d	17,50 b
2. Mineral Fertilizer	-	5.27 a	26.63 a	4,10 c	17,87 b
BSC	2	5.22 a	26.25 a	4,17 c	17,87 b
BSC	2.5	5.30 a	25.38 a	4,52 b	19,00 a
BSC	3	5.40 a	25.25 a	4,95 a	19,25 a
BSC	3.5	5.32 a	25.38 a	5,22 a	19,37 a
CV (%)		6.48	7.93	7,5	2,8
Average		5.21	25.75	4,45	18,47
----- Guarda Mor -----					
1. Control	-	4.50 c	20.25 a	3,38 a	14,92 c
2. Mineral Fertilizer	-	4.85 b	20.75 a	4,10 a	17,50 ab
BSC	2	4.75 b	20.75 a	3,68 a	16,95 b
BSC	2.5	4.82 b	20.50 a	3,38 a	16,75 b
BSC	3	4.85 b	21.13 a	3,45 a	16,60 b
BSC	3.5	5.42 a	21.63 a	3,93 a	18,65 a
CV (%)		4.96	5.78	15,6	6,84
Average		4.86	20.83	3,65	16,89

\* Means followed by the same letter in the columns do not differ by Duncan's test ( $p>0,10$ ); L ton<sup>-1</sup>: liters of the commercial product per ton of water; BSC: Biological Soil Conditioner; CV (%): coefficient of variation.

In Area 2 and Area 3, P leaf concentration was higher for treatments that received soil conditioner compared to the control and where only mineral fertilizer was applied in Area 2. The treatments increased the P grain concentration in Area 2 compared to control; in Area 3,

soil conditioner applied at 3 and 3.5 L ton<sup>-1</sup> were more efficient (Table 3). In Area 3, the soil conditioner applications at 2, 3, and 3.5 L ton<sup>-1</sup> were more efficient in increasing the K level in the grains. The positive nutritional results observed in soybean leaves and grains can be justified by the bacterial activity, which can solubilize and mineralize P from organic and inorganic sources [18] [19] [40] [41].

Phosphate solubilizing microorganisms play an important role in releasing inorganic forms of phosphorus (e.g., Ca-P, Al-P, Fe-P), increasing the P content in the soil solution, which provides higher levels of this element for plants, leaves, and grains. The mechanisms and ways that PGPB can solubilize phosphates are by producing and secreting organic acids, such as mono, di, and tri-carboxylic acids. These acids are a group of compounds that chelate cations (e.g., Al, Fe, Ca), such as gluconic acid, ketogluconic, ketogluconic, lactic acid, and acetic acid, and also by the action of glucose dehydrogenase. Many PGPB can solubilize phosphorus by acidification, chelation, or enzymatic processes [42] [43].

The differences between the studied areas regarding P concentration may be related to soil conditions and the soybean cultivar. In the area where P leaf concentration was lower (about 2.46 g kg<sup>-1</sup>), the plants were cultivated in soil with higher clay content (Area 2) compared to plants that were produced in sandy soil (Area 3) with higher average P leaf (about 5.21 g kg<sup>-1</sup>). It is essential to consider this factor since clay soils have higher P adsorption capacity on soluble fertilizers via soil and consequently influence P absorption and extraction by plants [44].

High doses of fertilizers are necessary to obtain satisfactory responses in soils with higher clay content. In Area 4, only the highest dose evaluated (soil conditioner at 3.5 L ton<sup>-1</sup>) presented higher P leaf concentration (5.42 g kg<sup>-1</sup>) and K grain concentration (18.65 g kg<sup>-1</sup>) of soybean. In addition to providing P, microorganisms can also act as nutrient solubilization (e.g., Fe, K, Zn) (Rodriguez et al., 2007) and therefore have favored the greater accumulation of K in grains.

Table 4 shows data on yield components, number of grains per pod, number of pods per plant, and weight of a thousand grains. In Area 1, pods with 1 and 4 grains were higher in plants that received soil conditioner at 3 L ton<sup>-1</sup>. However, the number of grains per pod and weight of a thousand grains did not differ among the treatments, with the number of pods per plant values ranging from 59.78 to 79.2 and the weight of a thousand grains ranging from 152.84 to 158.14 g.

**Table 4. Number of grains per pod (GP), number of pods per plant (NP), and weight of a thousand grains (WTG) as a function of the treatments applied to soybean cultivated in Uberlândia, Araxá, and Guarda Mor.**

Treatment	Dose (L ton <sup>-1</sup> )	GP				NP	WTG (g)
		1 grain	2 grains	3 grains	4 grains		
----- Uberlândia -----							
1. Control	-	1.42 bc*	10.83 a	46.18 a	1.35 c	59.78 a	152.85 a
2. Mineral fertilizer	-	0.77 c	12.85 a	56.18 a	1.73 bc	71.53 a	154.48 a
BSC	2	1.35 bc	14.00 a	54.28 a	1.25 c	70.88 a	152.84 a
BSC	2.5	2.00 ab	14.28 a	55.15 a	2.00 abc	73.43 a	154.03 a
BSC	3	2.62 a	17.13 a	56.15 a	2.83 a	78.73 a	157.59 a
BSC	3.5	2.10 ab	16.98 a	57.70 a	2.42 ab	79.20 a	158.14 a
CV (%)		63.49	31.74	24.97	52.48	23.01	3.98

Average		1.71	14.34	54.27	1.92	72.25	154.98
----- Araxá -----							
1. Control	-	3.30 a	12.00 a	25.90 b	0.175 a	41.37 b	179.94 a
2. Mineral Fertilizer	-	4.73 a	9.28 a	28.15 b	0.325 a	42.47 b	179.24 a
BSC	2	3.55 a	12.13 a	26.25 b	0.275 a	42.20 b	183.31 a
BSC	2.5	3.53 a	10.83 a	36.00 a	0.075 a	50.42 a	182.24 a
BSC	3	3.05 a	12.93 a	35.45 a	0.200 a	51.62 a	184.06 a
BSC	3.5	3.23 a	12.95 a	33.95 a	0.350 a	50.47 a	183.39 a
CV (%)		46.96	22.47	20.56	158.71	16.14	3.86
Average		3.56	11.68	30.95	0.233	46.42	182.02
----- Guarda Mor -----							
1. Control	-	4.75 a	23.75 a	33.53 a	0.134 a	62.16 a	129.83 c
2. Mineral Fertilizer	-	4.28 a	24.47 a	40.09 a	0.031 a	68.88 a	132.43 bc
BSC	2	4.16 a	25.13 a	35.25 a	0.219 a	64.75 a	132.75 bc
BSC	2.5	4.13 a	25.47 a	36.72 a	0.094 a	66.41 a	138.20 a
BSC	3	4.53 a	27.38 a	36.26 a	0.165 a	68.34 a	136.06 ab
BSC	3.5	4.59 a	26.94 a	40.34 a	0.125 a	72.00 a	134.77 ab
CV (%)		63.04	22.59	25.71	133.24	20.75	3.07
Average		4.40	25.52	37.03	0.127	67.08	134.01

\* Means followed by the same letter in the columns do not differ by Duncan's test ( $p > 0,10$ );  $L \text{ ton}^{-1}$ : liters of the commercial product per ton of water; BSC: Biological Soil Conditioner; CV (%): coefficient of variation.

The results of 3 grains per pod and NP observed in Area 3 showed that the use of soil conditioner at doses of 2.5, 3, and  $3.5 \text{ L ton}^{-1}$  was more efficient than the other treatments, although no statistical difference was found for WTG. These results are advantageous since soybean pods with 3 grains are the ones that contribute the most to increased productivity. The NP is the most important component when looking for increases in grain yield, which depends on the number of flowers emitted and fixed (not aborted) during the reproductive stage [46].

In Area 4, no significant difference ( $p > 0,10$ ) was observed for GP and NP. However, for WTG the soil conditioner treatment at  $2.5 \text{ L ton}^{-1}$  presented a significantly higher average (138.20 g). These results can be justified by the soil conditioner and the environment to which the plants are exposed; despite the soybean yield components being genetically predetermined, these factors (soil conditioner and environment) are determinants for the full genetic expression [47] [48] [49].

The treatment with mineral fertilizer and biological soil conditioner at the  $2.5 \text{ L ton}^{-1}$  dose presented significantly higher results for 1 grain per pod (Table 5). No significant differences were observed for 2 grains per pod and WTG.

**Table 5. The number of grains per pod (GP), number of pods per plant (NP), and weight of a thousand grains (WTG) as a function of the treatments applied to soybean cultivated in Paracatu**

Treatment	Dose ( $\text{L ton}^{-1}$ )	GP			NP	WTG (g)
		1 grain	2 grains	3 grains		
1. Control	-	0.93 b*	12.94 a	14.53 b	28.40 b	140.94 a
2. Mineral fertilizer	-	3.56 a	12.63 a	34.21 a	50.40 a	146.49 a
BSC	2	1.37 b	11.25 a	46.06 a	58.68 a	137.40 a
BSC	2.5	2.68 a	13.28 a	42.43 a	58.40 a	140.32 a
BSC	3	1.06 b	8.19 a	34.40 a	43.65 ab	135.68 a
BSC	3.5	1.59 ab	12.53 a	44.43 a	58.56 a	140.05 a

CV (%)	46.64	49.09	39.53	37	5.96
Average	1.86	11.8	36.01	49.68	140.14

\* Averages followed by the same letter in the columns do not differ by Duncan's test ( $p>0,10$ );  $L\ ton^{-1}$ : liters of the commercial product per ton of water; BSC: Biological Soil Conditioner; CV (%): coefficient of variation.

There was a significant effect of the treatments in the study on soybean yield (Table 6). The treatments that received mineral fertilizer and biological soil conditioner application were more efficient than the control of soybean yield in Area 1. The average grain yield achieved in Area 1 ( $4,624.8\ kg\ ha^{-1}$ ) was higher than that obtained in the state of Minas Gerais in the 2021/2022 harvest ( $3,828\ kg\ ha^{-1}$ ), according to CONAB [1].

**Table 6. Productivity due to the treatments applied to soybean crop grown in producing areas of Uberlândia, Paracatú, Araxá and Guarda Mor**

Treatment	Dose ( $L\ ton^{-1}$ )	( $kg\ ha^{-1}$ )	Productivity ( $sc\ ha^{-1}$ )	IR (%)
----- Uberlândia -----				
1. Control	-	4045.66 b*	67.43 b	-
2. Mineral fertilizer	-	4590.06 a	76.50 a	-
BSC	2	5104.03 a	85.07 a	11.20
BSC	2.5	4717.21 a	78.62 a	2.77
BSC	3	4668.04 a	77.80 a	1.69
BSC	3.5	4623.47 a	77.06 a	0.73
CV (%)		12.62	12.62	
Average		4624.74	77.08	
----- Paracatu -----				
1. Control	-	1241.06 c	20.68 c	-
2. Mineral Fertilizer	-	2923.83 b	48.73 b	-
BSC	2	3579.49 ab	59.66 ab	22.42
BSC	2.5	3459.75 ab	57.66 ab	18.32
BSC	3	3561.43 ab	59.36 ab	21.81
BSC	3.5	3864.01 a	64.40 a	32.15
CV (%)		25.78	25.78	
Average		3104.92	51.74	
----- Araxá -----				
1. Control	-	4601.09 c	76.68 c	-
2. Mineral Fertilizer	-	4812.59 bc	80.21 bc	-
BSC	2	5000.33 bc	83.34 bc	3.90
BSC	2.5	5292.39 ab	88.21 ab	9.97
BSC	3	5606.46 a	93.44 a	16.49
BSC	3.5	5304.38 ab	88.41 ab	10.22
CV (%)		11.49	11.49	
Average		5102.88	85.05	
----- Guarda Mor -----				
1. Control	-	3856.73 c	64.27 c	-
2. Mineral Fertilizer	-	4237.15 bc	70.61 bc	-
BSC	2	4117.18 bc	68.61 bc	-
BSC	2.5	4541.48 ab	75.69 ab	7.19
BSC	3	4568.81 ab	76.14 ab	7.83
BSC	3.5	4840.98 a	80.68 a	14.26
CV (%)		14.44	14.44	
Average		4360.39	72.67	

\* Averages followed by the same letter in the columns do not differ by Duncan's test ( $p>0,10$ ); L ton<sup>-1</sup>: liters of commercial product per ton of water; kg ha<sup>-1</sup>: kilograms per hectare; bag ha<sup>-1</sup>: bags per hectare (each bag = 60 kg); IR (%): increment relative compared to mineral fertilizer; BSC: Biological Soil Conditioner; CV: coefficient of variation.

In Area 2, the treatment of 3.5 L ton<sup>-1</sup> of soil conditioner resulted in higher productivity (3,864 kg ha<sup>-1</sup>), with increments of 2,620 and 940 kg ha<sup>-1</sup> compared to the control treatments and mineral fertilizer, respectively. Thus, in this assay, soybean yield was influenced by treatments with significant increments of 18.32 to 32.15% compared to mineral fertilizer (Table 6). The average yield achieved in the trial (3,104 kg ha<sup>-1</sup>) was practically equal to that obtained in the state of Minas Gerais in the 2020/21 crop season (3,016 kg ha<sup>-1</sup>), according to CONAB [1].

The same response was verified in Area 4. The soil conditioner dose of 3.5 L ton<sup>-1</sup> treatment of soil conditioner (4,841 kg ha<sup>-1</sup>) showed productivity increments of 985 and 604 kg ha<sup>-1</sup> compared to control and mineral fertilizer treatments, respectively (Table 6). The average yield achieved in the assay (4,360.2 kg ha<sup>-1</sup>) was higher than that obtained in Minas Gerais in 2020/21 crop season (3,828 kg ha<sup>-1</sup>), according to CONAB [1].

In Area 3, treatments affected soybean yield, which allowed gains of 3.90 to 16.49% of the use of soil conditioner compared to the mineral fertilizer (Table 6). The average yield achieved in Area 3 (5,103 kg ha<sup>-1</sup>) was higher than that obtained in the state of Minas Gerais in the 2020/21 crop (3,828 kg ha<sup>-1</sup>), according to CONAB [1]. These results corroborate those of Jain et al. [33] and Chagas Junior et al. [50] by showing that *Bacillus* sp. isolates can increase soybean yield.

The efficiency of plant growth promoting microorganism correlates with the soil biological activity [50]. Additionally, the relationship of microorganisms with soil is of great importance since soil characteristics can influence the efficiency of the soil conditioner. The results observed in the present study indicated that using *Bacillus*-based soil conditioner on soybean crops is a valuable strategy for more soybean productivity and sustainability of crop production.

#### 4. CONCLUSION

Overall, the data allows us to conclude that in medium texture soil (Area 1), the biological soil conditioner at 2 L ton<sup>-1</sup> was more efficient than the mineral fertilizer treatment to increase the P leaf concentration in soybean; on the other hand, in clay texture soil (Area 4), the most efficient biological soil conditioner dose was 3.5 L ton<sup>-1</sup>. Additionally, the use of biological soil conditioner at the 3 L ton<sup>-1</sup> was more efficient than mineral fertilizer to increase grain yield per pod in soybean cultivated in Area 1 and soybean yield (5,604 kg ha<sup>-1</sup>) grown in Area 3. Higher productivity was obtained at the biological soil conditioner dose of 3.5 L ton<sup>-1</sup> in Area 2 (3,864 kg ha<sup>-1</sup>) and Area 4 (4,836 kg ha<sup>-1</sup>).

This study revealed that using *Bacillus*-based soil conditioner on soybean crops is a valuable strategy to increase soybean yield and sustainability of agricultural activity.

#### REFERENCES

1. National Supply Company. Brazilian safra survey: grains, Safra 2021/2 Brazil: Conab, 2022. Accessed 29 March 2022. Available: <https://www.conab.gov>.

2. Timothy PB, Nurmiaty Y, Pramono E, Maysaroh S. (2020). Growth and yield responses of four soybean (*Glycine max* (L.) Merrill.) cultivars to different methods of NPK fertilizer application. *Tropical Plant: Journal of Agroscience*, 2020;8(1):39-43. <https://doi.org/10.18196/pt.2020.112.39-4>

[ PMC free article ] [ PubMed ] 3. Zhao S, Xu X, Wei D, Lin X, Qiu S, Ciampitti I, He P. (2020) Soybean Yield, Nutrient Uptake and Stoichiometry under Different Climatic Regions of Northeast China. *Scientific Reports*, 2020;10(1):1-9 <https://doi.org/10.1038/s41598-020-65447-6>

4. Susanna CS, Brunetto A, Marangon D, Tonello AA, Kulczynski SM. Influence of leaf fertilization on the quality of stored physiological soybean seed. *Biosphere Encyclopedia*, 2012;8(15):2385-2392. <https://www.researchgate.net/publication/282364582>.

5. Machado FR, Possenti JC, Fano A, Vismara ES, Deuner C. Soybean seed performance as a function of different seasonfoliar application of fertilizers. *Journal Behaviors*, 2020;16(31):107-122. <https://doi.org/10.31512/experiences.v16i31.217>.

6. Hungary M, Field RJ, Mendes IC. The importance of the biological nitrogen fixation process for soybean culture: an essential component for the competitiveness of Brazilian product. London : Embrap Soy , 2007 .

[ PMC free article ] [ PubMed ] 7. Rahman KMA, Zhang D. Effects of fertilizer broadcasting on the excessive use of inorganic fertilizers and environmental sustainability. *Sustainability*, 2018;10(1):759-771. <https://doi.org/10.3390/su10030759>.

8. Bhardwaj D, Ansari MW, Sahoo RK, Tujeta N. Biofertilizers function as a key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. *Microbial Cell Factories*, 2014;13(1):66-78. <https://doi.org/10.1186/1475-2859-13-66> .

9. Itelima JU, Bang WJ, Singing IA, Foot OJ. A review: biofertilizer; a key player in enhancing soil fertility and crop productivity. *Journal of Microbiology and Biotechnology Reports*, 2018;2(1):22-28. <https://doi.org/10.26765/DRJAFS.2018.4815>.

[ PubMed ] 10. Sumitra DB, Bamboriya JS, Shant I. Role of biofertilizers in agriculture - a review. *International Journal of Recent Scientific Research*, 2018;9(7):27727-27732. <http://dx.doi.org/10.24327/ijrsr.2018.0907.2319>.

[ PubMed ] 11. Coelho AF, Strap BO, Pears FF, Pereira SR. Evaluation of the foliar application of biofertilizer in four soy cultivars. *Trials and Science*, 2019;23(1):2-6. <https://doi.org/10.17921/1415-6938>.

12. Brazil. Decree No. 8,384, of 29 December 2014. Amends the Annex to Decree No. 4,954, of 14 January 2004, approving the Regulation of Law No. 6,894, of 16 December 1980, providing for inspection and supervision of the production and trade of fertilizers, correctives, inoculants or biofertilizers intended for agriculture. *Official Journal of the Union, Brasilia, DF, Session 1, 24p.*,

[ PubMed ] 13. Backer R, Rokem JS, Ilangumaran G, Lamont J, Praslickova D, Ricci E, Subramanian S, Smith DL. Plant growth-promoting rhizobacteria: Context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. *Frontiers in Plant Science*, 2018;9(1):1473-1489. <https://doi.org/10.3389/fpls.2018.01473> .

[ PMC free article ] [ PubMed ] 14. Basu A, Prasad P, Das SN, Kalam S, Sayyed RZ, Reddy MS, Enshasy H. Plant growth promoting rhizobacteria (PGPR) as green bioinoculants: recent developments, constraints, and prospects. *Sustainability* 2021;13(1):1140-1149. <https://doi.org/10.3390/su1>

[ PMC free article ] [ PubMed ] 15. Oleska E, Małek W, Wójcik M, Swiecicka I, Thijs S, Vangronsveld J. Beneficial features of plant growth-promoting rhizobacteria for improving plant growth and health in challenging conditions: A methodical review. *Science of the Total Environment*, 2020;15(1) 743-752. <https://doi.org/10.1016/j.scitotenv.2020.140682>.

[ PMC free article ] [ PubMed ] 16. Merdia B, Rokaia BM, Asmaa B. Biological control by plant growth promoting rhizobacteria. *Algerian Journal of Biosciences*, 2020;1(2):30-36. <http://dx.doi.org/10.5281/zenodo.4393567>.

[ PubMed ] 17. Jiao X, Takishita Y, Zhou G, Smith DL. Plant Associated Rhizobacteria for Biocontrol and Plant Growth Enhancement. *Frontiers in Plant Science*, 2021;12(6)347-356. <https://doi.org/10.3389/fpls.2021.634796>.

[ PMC free article ] [ PubMed ] 18. Contreras-Cornejo HA, Macías-Rodríguez L, Val E, Larsen J. Eco-logical functions of *Trichoderma* spp. and their secondary metabolites in the rhizo-sphere: interactions with plants. *FEMS Microbiology Ecology*, 2016;92(1):1-17. <https://doi.org/10.1093/femsec/fiw036>

[ PubMed ] 19. Zeilinger S, Sabine G, Ravindra B, Prasun KM. Secondary metabolism in *Trichoderma* – Chemistry meets genomics. *Fungal Biology Reviews*, 2016;30(2):74-90. <https://doi.org/10.1016/j.fbr.2016.05.001>.

[ PubMed ] 20. Sagar A, Yadav SS, Sayyed RZ, Sharma S, Ramteke PW. *Bacillus subtilis*: A multifarious plant growth promoter, biocontrol agent, and bioalleviator of abiotic stress. Cham: Springer, 2022. [https://doi.org/10.1007/978-3-030-85465-2\\_24](https://doi.org/10.1007/978-3-030-85465-2_24).

21. Lanna Filho R, Ferro HM, Pinho RSC. Biological control mediated by *Bacillus subtilis*. *Tropical Journal: Agricultural and Biological Sciences*, 2010;4(2):12-20. <https://doi.org/10.0000/rtcab.v4i2.145>.

[ PubMed ] 22. Lima ODR, Oliveira LJMG, Silva MSBS, Rodrigues AAC. In vitro antifungal activity of *Bacillus* sp. isolated on *Fusarium oxysporum* sp. *lycopersici*. *Coating Journal*, 2014;27(4):57-64.

[ PMC free article ] [ PubMed ] 23. Zhao Y, Selvaraj JN, Xing F, Zhou L, Wang Y, Song H, Tan X, Sun L, Sangare L, Folly YME, Liu Y. Antagonistic action of *Bacillus subtilis* strain SG6 on *Fusarium graminearum*. *PLOS ONE*, 2014;9(3)1-11. <https://doi.org/10.1371/journal.pone.0092486>.

[ PMC free article ] [ PubMed ] 24. Calvo P, Ormeño-Orrillo E, Martínez-Romero E, Zuñiga D. Characterization of *Bacillus* isolates of potato rhizosphere from Andean soils of Peru and their potential PGPR characteristics. *Brazilian Journal of Microbiology*, 2010;41(4):899-906. <https://doi.org/10.1590/S1517-83822010000400008>.

[ PubMed ] 25. Gagné-Bourque F, Mayer BF, Charron J, Vali H, Bertrand A, Jabaji S. Accelerated growth rate and increased drought stress resilience of the model grass *Brachypodium distachyon* colonized by *Bacillus subtilis* B26. *PLOS ONE*, 2015;10(6):1-23. <https://doi.org/10.1371/journal.pone.0130456>.

[ PubMed ] 26. Kavamura VN, Santos SN, Silva JL, Parma MM, Avila LA, Visconti A, Zucchi TD, Taketani RG, Andreote FD, Melo IS. Screening of Brazilian cacti rhizobacteria for plant growth promotion under drought. *Microbial Research*, 2013;168(4):183-191. <https://doi.org/10.1016/j.micres.2012.12.002>.

[ PubMed ] 27. Kundan R, Pant G, Jadon N, Agrawal PK. Plant growth promoting rhizobacteria: mechanism and current perspective. *Journal of Fertilizers and Pesticides*, 2015;6(2):1-9. <https://doi.org/10.4172/2471-2>

[ PubMed ] 28. Radhakrishnan R, Hashem A, Abdallah EF. Bacillus: a biological tool for crop improvement through bio-molecular changes in adverse environments. *Frontiers in Physiology*, 2017;8(1):1-8. <https://doi.org/10.3389/fphys.2017.00667> .

[ PubMed ] 29. Braga Junior GM, Colony BSO, Chagas LFB, Scheidt GN, Miller LO, Chagas Junior AF. Efficiency of inoculation by *Bacillus subtilis* on soybean biomass and productivity. *Brazilian Journal of Agricultural Sciences*, 2018;13(4):55-71. <https://doi.org/10.5039/agrarian.v13i4a5571>.

[ PubMed ] 30. Diaz PAE, Baron NC, Rigobelo EC. *Bacillus* spp. as plant growth-promoting bacteria in cotton under greenhouse conditions. *Australian Journal of Crop Science*, 2019;13(12):2003-2014. <https://doi.org/10.21475/ajcs.19.13.12.p2003>.

[ PubMed ] 31. Hashem A, Tabassum B, Abdallah EF. *Bacillus subtilis*: A plant-growth promoting rhizobacterium that also impacts biotic stress. *Saudi Journal of Biological Sciences*, 2019;26(6):1291-1297. <https://doi.org/10.1016/j.sjbs.2019.05.004>.

[ PMC free article ] [ PubMed ] 32. Dame ZT, Rahman M, Islam T. Bacteria as sources of agrobiotechnology: recent advances and future directions. *Green Chemistry Letters and Reviews*, 2021;14(2):246-271. <https://doi.org/10.1080/17518253.2021.1905080>.

[ PubMed ] 33. Jain S, Vaishnav A, Choudhary DK, Sharma PK. Isolation and characterization of plant growth promoting bacteria from soybean rhizosphere and their effect on soybean plant growth promotion. *International Journal of Advanced Scientific and Technical Research*, 2016;5(1):397-410. Retrieved from <http://www.rspublication.com/ijst/index.html>.

[ PMC free article ] [ PubMed ] 34. Shafi J, Tian H, Ji M. *Bacillus* species as versatile weapons against plant pathogens: a review. *Biotechnology & Biotechnology Equipment*, 2017;1(1):446-459. <https://doi.org/10.1080/13102818.2017.1286950>.

[ PubMed ] 35. Santos HG, Jacomine PKT, Anjos LHC, Oliveira VA, Lumbreras JF, Coelho MR, Almeida JA, Araujo Filho JC, Oliveira JB, Cunha TJF. Brazilian system of classification of singles. 3rd ed., Embrapa: Rio de Janeiro, 2017.

[ PubMed ] 36. Ribeiro AC. Recommendations for the use of correctives and fertilizers in Minas Gerais: 5th ed., Belo Horizonte: SBCS, 359 p.

37. Brazilian Agricultural Research Company. Manual of chemical analyzes of singles, plants and fertilizers. 2 ed. rev. and expanded. Brazil: Embrapa, 627 p.,

[ PubMed ] 38. Bataglia OC, Furlani AMC, Teixeira JPF, Furlani PR, Gallo JR. Methods of chemical analyzes of plants. Campinas: Agronomic Institute of Campinas. 48 p.,

[ PubMed ] 39. Canteri MG, Althaus RA, Virgins Son JS, Giglioti EA, Godoy CV. SASM-Agri System for analysis and separation of media in agricultural experiments by Scott-Knott, Tukey and Duncan methods. London: Embrapa, 2001.

[ PubMed ] 40. Richardson AE. Prospects for using soil microorganisms to improve phosphorus acquisition by plants. *Australian Journal of Plant Physiology*, 2001;28(1):897-906. <https://doi.org/10.1071/PP01093> .

[ PMC free article ] [ PubMed ] 41. Elhaisoufi W, Ghoulam C, Barakat A, Zeroual Y, Bargaz A. Bacterial solubilization of phosphate: A key rhizosphere driving force enabling higher P use efficiency and crop productivity. *Journal of Advanced Research*. 2022;38(5):13-2 <https://doi.org/10.1016/j.jare.2021.08.014>.

42. Perez-Montano F, Alias-Villegas C, Bellogin RA, Hill P, Espuny MR, Jimenez-Guerrero I, Lopez-Baena FJ, Ollero FJ, Cube T. Plant growth promotion in cereal and leguminous agricultural important plants: from microorganism capacities to crop production. *Microbiological Research*, 2014;169(5):325-336. <https://doi.org/10.1016/j.micres.2013.09.011>.

43. Denaya S, Yulianti R, Pambudi A., Effendi Y. Novel microbial consortium formulation as plant growth promoting bacteria (PGPB) agent. *IOP Conf. Series: Earth and Environmental Science*, 2021;637(1):12-30. <https://doi.org/0.1088/1755-1315/637/1/012030>.

44. Nicchio B, Korndörfer GH, Pereira HS, Neto AG. Effect of the mixture of acidulated phosphates, natural phosphates and sulfur sources on the growth and phosphorus and sulfur uptake of sugarcane. *Journal of Plant Nutrition*, 2022;45(5):775-788. <https://doi.org/10.1080/01904167.2021.1985135>.

45. Rodriguez H, Fraga R, Gonzalez T, Bashan Y. Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. *First International Meeting on Microbial Phosphate Solubilization*, Netherlands: Springer, 2007.

46. Mundstock CM, Thomas AL. Soja: fatores que afetam o crescimento e o rendimento de grãos. Porto Alegre: Universidade Federal do Rio Grande do Sul. 37 p., 2005.

47. Singh SK, Barnaby JY, Reddy VR, Sicher RC. Varying response of the concentration and yield of soybean seed mineral elements, carbohydrates, organic acids, amino acids, protein, and oil to phosphorus starvation and CO<sub>2</sub> enrichment. *Frontiers in Plant Science*, 2016;7(1):1967-1979. <https://doi.org/10.3389/fpls.2016.01967>.

48. He J, Jin Y, Du YL, Wang T, Turner NC, Yang RP, Siddique KHM, Li FM. Genotypic variation in yield, yield components, root morphology and architecture, in soybean in relation to water and phosphorus supply. *Frontiers in Plant Science*, 2017;8(1):1499-1507. <https://doi.org/10.3389/fpls.2017.01499>.

49. Savala C, Wiredu A, Okoth J, Kyei-Boahen S. Inoculant, nitrogen and phosphorus improves photosynthesis and water-use efficiency in soybean production. *The Journal of Agricultural Science*, 2021;159(5):349-362. <https://doi.org/10.1017/S0021859621000617>.

50. Chagas Junior AF, Braga Junior GMBJ, Lima CAL, Martins ALLM, Souza MCS, Chagas LFBC. *Bacillus subtilis* as a vegetable growth promoter inoculant in soybean. *Diversitas Journal*, 2022;7(1):1-16. <https://doi.org/10.48017/dj.v7i1.2071>.