Article

Influence of *Bacillus* sp. on soil chemical and microbiological attributes and development of soybean and maize

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Abstract

Plant growth-promoting rhizobacteria (PGPR) inhabit the rhizosphere of several cultivated plants. Bacteria of the genus Bacillus have great functional importance in plant development. The objective of this research was to evaluate different doses of *Bacillus* sp. in the development of soybean and corn crops and in the chemical and microbiological attributes of the soil. The essays were conducted in the field in the 2016/2017 harvest. The soybean and corn seeds were treated with two doses of *Bacillus* sp. at a concentration of 10^9 CFU ml^{-1}, with 10 and 20 ml of 100 kg of seed used in soybeans and 80 and 100 ml of 100 kg of seed in corn. The experiments were completely randomized in block with four repetitions. Chemical and microbiological attributes and plant development were evaluated. The results showed that the microbial activity was achieved at the dose of 20 ml of *Bacillus* sp. and positively influenced the weight of the grains and soybean productivity. In corn crops, the dose of 80 ml of *Bacillus* sp. showed better performance on chemical and microbiological attributes, resulting in increased shoot and root mass, and a significant increase in productivity. From the results obtained, it can be concluded that the use of *Bacillus* sp. it is a viable alternative for sustainable agriculture, and the dose of 20 ml and 80 ml of 100 kg of soybean and corn seeds, respectively, presented better results in microbial activity and in the development of plants.

Keywords: *Glycine max*, *Zea mays*, inoculants, plant growth promotion rizobacteria, soil quality of bioindicators.

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Introduction

Soybeans and corn cultivation are growing agricultural activities in the world and Brazil stands out with record production of 120.9 million tons of soybeans and 100.9 million tons of corn in the 2019/2020 harvest (CONAB, 2020). These crops are important both for the production of animal feed, oils and fuel, and for human food (CONAB, 2020). Due to population growth, the demand for food production requires the development of new technologies applied to crops aiming to improve the quality and increase of production of grains (Ratz *et al*., 2017; Buchelt *et al*., 2019).

Success in establishing a crop depends on the soil and the environment, which must be suitable for seed germination, seedling emergence and development (Modolo *et al*., 2011). In addition, the cultivation of soybeans and corn depends on the propagation material, which is correctly selected, with high germination power, excellent vigor and without contamination with pests and diseases (Migliorini *et al*., 2017).

To increase productivity and decrease costs for the producers, alternatives to reduce the use of inputs and pesticides have been sought. An effective alternative is the use of plant growth promoting rhizobacteria (PGPR), which is a promising biological option in the control of phytopathogens, plant development and in increasing crop productivity (Hernández-Hernández *et al*., 2018). PGPR are bacteria that are found in the rhizosphere, root surface and in association with roots and are often isolated from the rhizosphere of several cultivated plants, to promote growth (Ratz *et al*., 2017). *Bacillus*, *Pseudomonas*, *Azospirillum*, *Rhizobium*, *Serratia*, *Azotobacter* among others are among the most studied genera (Araújo and Guerreiro, 2010). The benefits observed in several cultures with the inoculation of bacteria of the genus *Bacillus*, show its great potential as biological control agents, production of natural antibiotics and protective effect against soil phytopathogens. These organisms support growth and development of the root system, greater absorption of water and nutrients, favoring the development of the crop, leading to a quality production of its seeds (Ferreira *et al*., 2018; Machado and Costa, 2018). The use of *Bacillus*, or its metabolites, can contribute with phytohormones and molecular signals, increasing root growth, nodulation and N_2 fixation rates, and reducing susceptibility to water stress (Ferreira *et al*., 2018; Machado and Costa, 2018; Buchelt *et al*., 2019).

The use of *Bacillus* spp., species as growth promoters for several agricultural crops provides an attractive, efficient and less aggressive method compared to pesticides and chemical fertilizers This makes the practice of using bioagents a more sustainable alternative from an economic and environmental standpoint (Shafi *et al*., 2017). Several species of PGPRs have been increasingly studied and their application intensified to increase productivity. However, any and all anthropic activities, whether cultural management or the introduction of products and organisms into the soil, can cause impact or stress to the soil microbial community responsible for nutrient mineralization and decomposition. Thus, the use of soil quality indicators (bioindicators) is sensitive to any change or management of the soil (Simão *et al*., 2020).

In this study, two field trials were carried out in order to evaluate the influence of seed treatments with two doses of *Bacillus* spp., on the chemical and microbiological attributes of the soil and on the development of soybean and corn crops.

Material and methods

The tests were conducted during the 2016/2017 crop year, at the farm school of the State University of Northern Paraná (S23º 06' 24.7" and W50º 21' 37.36" and 439 m altitude), *Campus* Luiz Meneghel (CLM), located in the municipality of Bandeirantes, Paraná, Brazil. The soil in the experimental area is classified as Eutropheric RED Latosol (EMBRAPA, 2006). The climate of the region is classified as Cfa type (Koppen and Geiger), humid subtropical. During the months of the experiments (October 2016 to February 2017) the maximum average temperature was 28 ºC and the minimum 18 ºC, and the average monthly rainfall was 200 mm (Center for Weather Forecast and Climate Studies-CPTEC). The tests were conducted with soybean (*Glycine max* L.) variety BMX Potencia and corn (*Zea mays* L.) variety KWS 9004 in randomized block with four replications.

Inoculated bacteria

The bacterium *Bacillus* sp., evaluated in this study was isolated from soil samples collected at Farm School of the State University of Northern Paraná-CLM. Planting area for agricultural crops with application of biological fertilizer for three consecutive years.

Cultivation and inoculation of *Bacillus* sp. in soy and corn seeds

Bacillus sp., was isolated and grown in nutrient broth and kept in BOD at 28 ºC until the bacterial population reached the concentration of $1x10^9$ CFU ml^{-1} determined by a spectrophotometer at 530 nm, according to Normative Instruction No. 13 of the Agricultural Defense Department of March 24 of 2011. The soybean and corn seeds were inoculated approximately ten minutes before sowing. The seeds were inoculated with two doses of the bacterial suspension, the proposed commercial dose and double the dose (Table 1). For both cultures, 2 kg of seed were treated with *Bacillus* sp. in a concentration of $1x10^9$ CFU ml^{-1} homogenized in plastic bags, transferred in paper bags and kept in the shade until sowing.

Table 1. Description of treatments (doses and concentration) of the inoculation tests of soybean and corn seeds with *Bacillus* sp.

Treatments	Bacterium	Concentration (CFU ml^{-1})	Dose (ml 100 kg^{-1} of seeds)	
			Soybean	Corn
T1	Without inoculation	-	-	-
T2	*Bacillus* sp.	$1x10^9$	10	80
T3	*Bacillus* sp.	$1x10^9$	20	100

CFU= colony forming unity.

Experiment's installation

The cultivation of soybeans and corn were installed in the field in the no-tillage system and the seeds were sown with the aid of a bicycle planter. The soybean was sown at a density of 13 seeds per meter and 45 cm spacing between rows. Together with the sowing, a basic fertilization was carried out with 300 kg ha^{-1} of the formulated fertilizer (NPK) 0-10-10. The corn was sown at the density of three seeds per linear meter and with a spacing of 50 cm between the lines. Basic fertilization was carried out with 250 kg ha^{-1} of the formulated fertilizer (NPK) 10-18-18.

The experimental design was a randomized block, with three treatments and four replications for each crop (soybean and corn), each plot measuring 5 m wide and 6 m long, making an area of 30 m^2. The treatments consisted of control and two doses of *Bacillus* sp., at a concentration of 10^9 CFU ml^{-1} (Table 1). Soil collection for chemical and microbiological analysis was carried out post-harvest at a depth of 0-10 cm, with 7 samples being collected to compose a sample made up of a plot, which were homogenized, packed in plastic bags and transported in a thermal box to the Soil Microbiology Laboratory, in the State University of Northern Paraná, *Campus* Luiz Meneghel, where they were separated from plant and animal waste and sieved in a 2 mm mesh.

Soil chemical analysis

After air drying the soil samples, the pH was determined in 0.01 M $CaCl_2$, P, Ca^{2+}, Mg^{2+}, K^+ and Al^{3+}. The contents of Ca^{2+}, Mg^{2+}, K^+ and Al^{3+} were extracted with 1 M KCl and determined by atomic absorption (Ca^{2+} and Mg^{2+}) and titrated with 0.025 M NaOH (Al^{3+}); P and K^+ were extracted with Mehlich-1 extractor and determined by flame ionization spectrophotometry (K^+) and by the molybdenum blue method (P).

Soil microbiological analysis

Microbial biomass Carbon (MBC). The carbon content from microbial biomass of the soil was determined by the fumigation-indirect extraction (FIE) method (Vance *et al*., 1987). Basal respiration (BRS) and soil metabolic quotient (qCO_2) determination. Basal soil respiration and qCO_2 were determined according to the methodology proposed by Silva *et al*. (2010).

Total organic carbon (TOC) and microbial quotient (qMIC). TOC determination was carried out in combustion of organic matter via wet, using 0.5 g of sample, according to Walkley and Black (1934-modified), without external heating in the plate. The qMIC was determined by the MBC/TOC ratio.

Agronomic analysis

The root height and length evaluation was determined from 10 plants chosen at random. The dry shoot and root mass, being sampled 5 plants per plot. The soybean yield was determined by harvesting four linear meters per plot and the weight of 1 000 grains was also obtained. Corn productivity was determined by harvesting two linear meters per plot, obtaining a weight of 200 grains. At the time of harvest, the grains (soybeans and corn) were at 13% moisture. The productivity results were determined in kg ha^{-1}.

All statistical analyses were carried out using Sisvar (version 5.7, DEX/UFLA). The averages were submitted to Anova. When confirming a statistically significant *p* value, the Tukey test ($p < 0.05$) was applied for comparison purposes (Ferreira, 2019).

Results and discussion

In soybean cultures, chemical analysis showed that inoculation of *Bacillus* sp., regardless of the dose, resulted in a significant reduction in pH, organic matter and potassium content compared to the control (Table 2). This may be related to the degradation of organic matter by bacteria, which can oxidize a wide range of organic and fermentative compounds (Stamford *et al*., 2005). Plant growth-promoting rhizobacteria can help accelerate the decomposition of organic matter as in the mineralization process (Persello-Cartineaux *et al*., 2003). Organic matter serves as a source of carbon and energy for microorganisms, because a high content of organic material is associated with a great microbial diversity (Wetler-Tonini *et al*., 2010).

In T2, a slight increase in phosphorus content was observed. Studies carried out with *Bacillus subtilis* have shown greater availability of nutrients in the soil, indicating greater absorption of P and N, in plant inoculated with PGPRs in the seeds (Araújo, 2008). The increase may also be related to the no-tillage method, caused by less soil mobilization, in addition to the formation of phosphorus complexes with organic matter, greater biological activity and recycling of nutrients by the roots and crop residues.

Table 2. Chemical analysis of the soil carried out post-harvest of soybean, from areas with and without inoculation of *Bacillus* sp.

Treat	OM	pH	P	K	Ca	Mg	Al	H+Al	CEC	V
	(g kg^{-1})	($CaCl_2$)	(mg dm^{-3})	(mol$_c$ dm^{-3})						(%)
T1	33.24 a	5.15 a	5.5 a	0.64 a	6.48 a	2.78 a	0 b	4.66 a	14.55 ab	68.03 a
T2	28.54 b	4.88 b	8.29 a	0.41 b	6 a	3.18 a	0.18 a	5.28 a	14.86 a	64.5 a
T3	27.53 b	5.03 ab	4.84 a	0.32 b	5.8 a	2.85 a	0.03 b	4.65 a	13.61 b	65.76 a
CV (%)	2.38	2.53	38.91	18.49	9.86	8.39	61.24	8.46	4.02	4.16

Data: OM= organic matter; P= phosphorus; K= potassium; Ca= calcium; Mg= magnesium; Al= aluminum; H+Al= potential acidity; CEC= cation exchange capacity; V%= base saturation; T1= control without bacteria; T2= *Bacillus* sp., 10 mL 100 kg^{-1} seed; T3= *Bacillus* sp., 20 ml 100 kg^{-1} seed. Averages followed by the same lowercase letter in the column, do not differ by Tukey's test at 5% probability.

In corn, chemical analyzes showed less change in the parameters evaluated, with only a drop in the content of P and K observed with the inoculation of *Bacillus* sp. (Table 3). This is likely related to rhizobacteria promoting the mineralization of nutrients, solubilization of phosphates, nitrogen fixation and increased absorption of nutrients by the roots (Lazarovits and Nowak, 1997).

In soybean cultures, microbiological parameters showed a decrease in TOC content in treatments inoculated with the bacteria (T2 and T3). This may be related to the increase in microbial biomass, being significantly higher in T3 (154.66) when compared to the others treatments (Table 3).

Table 3. Chemical analysis of the soil carried out post-harvest of soybean, from areas with and without inoculation of *Bacillus* sp.

Treat	OM ($g\ kg^{-1}$)	pH ($CaCl_2$)	P ($mg\ dm^{-3}$)	K	Ca	Mg	Al	H+Al	CEC	V (%)
				($mol_c\ dm^{-3}$)						
T1	21.82 a	5.03 a	31.35 a	0.74 a	5.7 a	3.33 a	0.05 a	7.2 a	16.97 a	58.22 a
T2	24.51 a	5.03 a	22.44 b	0.4 b	6.4 a	3.28 a	0.03 a	6.3 a	16.37 a	61.52 a
T3	24.17 a	4.95 a	16.11 b	0.41 b	6.55 a	2.33 a	0.08 a	5.69 a	14.97 a	61.88 a
CV (%)	15.42	3.92	42.82	13.13	21.97	24.1	141.4	23.36	6.31	11.95

OM= organic matter; P= phosphorus; K= potassium; Ca= calcium; Mg= magnesium; Al= aluminum; H+Al= potential acidity; CEC= cation exchange capacity; V%= base saturation; T1= control without bacteria; T2= *Bacillus* sp., 10 ml 100 kg^{-1} seed; T3= *Bacillus* sp., 20 ml 100 kg^{-1} seed. Averages followed by the same lowercase letter in the column, do not differ by Tukey's test at 5% probability.

In addition to the significant increase in MBC in T3, there was a better rate of decomposition and mineralization of organic matter in the soil (*q*MIC), as well as lower respiration (SBR) and lower metabolic stress (qCO_2) (Table 3). In the corn crops, the best values for microbiological parameters were observed in the T2 treatment, with significantly higher microbial biomass (175.87) and higher *q*MIC (1.25). Consequently, less metabolic stress was observed (2.26) (Table 4). Microbial biomass (BM) is the living part of the soil, and it acts on the decomposition of organic matter in the soil. The amount and composition of microbial biomass can be influenced by several factors, including the cultivation system, crop rotation and soil texture (Venzke-Filho *et al*., 2008).

Table 4. Analysis of soil microbiological attributes in soybean culture with and without inoculation of *Bacillus* sp.

Treat	TOC ($g\ kg^{-1}$)	MBC ($mg\ C\ kg^{-1}$)	*q*MIC (%)	SBR ($mg\ C\text{-}CO_2\ kg^{-1}\ h^{-1}$)	qCO_2 SBR C_MBS^{-1}
T1	19.28 a	73.62 b	0.38 b	0.46 ab	6.33 b
T2	16.55 b	83.83 b	0.51 b	0.71 a	8.53 a
T3	15.97 b	154.66 a	0.97 a	0.36 b	2.29 c
CV (%)	2.38	12.75	12.99	24.52	18.71

TOC= total organic carbon; MBC= microbial biomass carbon; (*q*MIC)= microbial quotient; (SBR)= soil basal respiration; (qCO_2)= soil metabolic quotient. T1= control; T2=*Bacillus* sp., 10 ml 100 kg^{-1} seed; T3 = *Bacillus* sp. 20 ml 100 kg^{-1} seed. Averages followed by the same lowercase letter in the column, do not differ by Tukey's test at 5% probability.

The microbial quotient (*q*MIC) is equivalent to the percentage of reserve of total organic carbon in the soil, considered as equilibrium soil values between 1.8 to 2.2% (Jakelaitis *et al*., 2008). Areas with low microbial activity present lower values, demonstrating loss of carbon in the soil, which indicates a lower availability of organic compounds to plants (Simão *et al*., 2020). Variations in *q*MIC values reflect the pattern of soil organic matter input, the efficiency of microbial C conversion, soil C losses and the stabilization of organic C by soil mineral fractions (Cunha *et al*., 2011).

As the microbial biomass becomes more efficient in the use of ecosystem resources, less CO_2 is lost through respiration and the greater the proportion of C incorporated into microbial tissues, resulting in a decrease in qCO_2 in the soil (Cunha *et al*., 2011). Estimates of microbial biomass have been used in studies of C and N flow, nutrient cycling and plant productivity in different terrestrial ecosystems, also enabling the association of the amount immobilized nutrients and the activity of microbial biomass with fertility and the potential for soil productivity (Gama-Rodrigues *et al*., 2008). In the study by Chagas *et al*. (2017), with soybeans and cowpea, the plants showed superior results in terms of biomass accumulation, demonstrating the potential of *Bacillus* sp., as a growth promoter for both cultures.

The agronomic attributes evaluated in the soybean culture did not show significant differences between the treatments, however, there was a trend of greater plant height, greater root length and, consequently, greater productivity in the T3 treatment (Table 6). This can be related to the increase in microbial biomass and microbial quotient (Table 5).

Table 5. Analysis of soil microbiological attributes in corn culture with and without *Bacillus* sp.

Treat	TOC ($g\ kg^{-1}$)	C-MBS ($mg\ C\ kg^{-1}$)	*q*MIC (%)	SBR mg $C\text{-}CO_2$ ($kg^{-1}\ h^{-1}$)	qCO_2 SBR C-MBS^{-1}
T1	12.66 a	98.32 b	0.78 b	0.26 b	2.66 b
T2	14.22 a	175.87 a	1.25 a	0.4 ab	2.26 b
T3	14.02 a	91.77 b	0.67 b	0.47 a	5.17 a
C.V. (%)	15.42	11.43	14.8	24.9	26.89

TOC= total organic carbon; MBC= microbial biomass carbon; *q*MIC= microbial quotient; SBR= soil basal respiration; (qCO_2)= soil metabolic quotient; T1= control; T2= *Bacillus* sp., 10 ml 100 kg^{-1} seed; T3= *Bacillus* sp., 20 ml 100 kg^{-1} seed. Averages followed by the same lowercase letter in the column, do not differ by Tukey's test at 5% probability.

Table 6. Parameters of soybean development (shoot and root) and yield after inoculation of the seeds with *Bacillus* sp., in two doses.

Treat	Shoot		Root		Yield	
	H(cm)	FSM(g)	RL(cm)	DRM(g)	W1000(g)	kg ha^{-1}
T1	28.1 a	27.46 a	20.15 a	24.27 a	127.5 b	2.98 a
T2	28.13 a	27.69 a	20.7 a	25.71 a	128.57 ab	3.096 a
T3	29 a	27.25 a	23.6 a	25.35 a	134.28 a	3.249 a
CV(%)	4.07	8.16	8.16	5.96	2.50	8.45

H= plant height; FSM= fresh shoot mass; RL= root length; DRM= dry root mass; W1 000= weight 1 000 grains; kg ha^{-1}= kilogram per hectare. T1= control; T2= *Bacillus* sp., 10 ml 100 kg^{-1} seed; T3= *Bacillus* sp., 20 ml 100 kg^{-1} seed. Averages followed by the same lowercase letter in the column, do not differ by Tukey's test at 5% probability.

Bacteria in natural habitats colonize the interior and exterior of plant organs and that can beneficial, neutral or harmful to their growth (Mariano *et al*., 2004). Domenech *et al*. (2006) demonstrated that *Bacillus* sp., auxin producers improved the development of the plant, increasing the height and fresh weight of the aerial part. This increase allowed a better photosynthetic rate and, consequently, a better efficiency in the use of water and an increase in production (Szilagyi-Zecchin *et al*., 2015).

Plants that have microorganisms associated with their roots or in the rhizosphere tend to have a better ability to survive and consequently better absorption of nutrients. Thus, plants that are assured of beneficial factors by microorganisms have a productive advantage over those that do not (Chagas *et al*., 2017).

In corn, the benefits of inoculation with Bacillus sp., was more evident, showing greater plant height and dry matter, both in shoot and root. However, productivity was significantly higher in the T2 treatment (6.026 kg ha^{-1}) (Table 7). Moreira (2014) highlighted the specificity of the association between the bacterium *Bacillus* spp., and the plant since the plants genotype is a determining factor for obtaining the benefits from inoculation.

Table 7. Parameters of corn development (shoot, root and yield), with inoculation of *Bacillus* sp., in two doses.

Treat	Shoot		Root		Yield	
	H (cm)	FSM (g)	RL (cm)	DRM (g)	W200 (g)	(kg ha^{-1})
T1	69.25 b	104.02 b	25.55 a	34.68 b	68.44 a	5.024 b
T2	83.75 a	125.75 a	23.55 a	39.6 a	69.42 a	6.026 a
T3	81.35 a	124.84 a	22.55 a	38.32 ab	67.27 a	4.885 b
CV (%)	5.7	4.69	34.26	5.86	4.25	4.34

H= plant height; FSM= fresh shoot mass; RL= root length; DRM= dry root mass; W200= weight 200 grains; kg ha^{-1}= kilogram per hectare. T1= control; T2= *Bacillus* sp., 10 mL 100 kg^{-1} seed; T3=*Bacillus* sp., 20 mL 100 kg^{-1} seed. Averages followed by the same lowercase letter in the column, do not differ by Tukey's test at 5% probability.

Bacillus sp., promotes growth and yield of different crops, in addition to improving the nutrient absorption capacity, resulting in more vigorous plants (Shafi *et al*., 2017). Dotto *et al*. (2010); Rodrigues *et al*. (2006), reported contrasting productivity results of different maize hybrids with bacterial application. Lima *et al*. (2011), reported positive results in the development of plant shoot and root, improving its establishment and consequently the grain yield in his research of corn seeds with *Bacillus* spp., sown in soil that received NPK fertilization.

The success of *B. Subtilis* in promoting plant growth is related to the biological characteristics of these microorganisms, which are easy to maintain (Lanna-Filho *et al*., 2010). The growth promotion caused by *Bacillus* spp., is the result of increased nitrogen fixation, solubilization of nutrients, synthesis of phytohormones and improved soil conditions (Lanna-Filho *et al*., 2010).

Conclusions

The inoculation of *Bacillus* sp., demonstrated different results in soybean and corn crops. In soybeans, the application of *Bacillus* sp., showed better results in microbiological attributes in the highest dose, (twice the commercial dose). In the other attributes there were no major changes. It is important to note that bacteria inoculation does not cause damage in the culture production. This result will be further investigated in the future, with the objective of developing a commercial product based on *Bacillus* sp., exposing a positive evolution in plant development and yield.

In the studied corn crops, the inoculation of *Bacillus* sp. resulted in an increase in plant shoot, root and yield, in the commercial dose, with great importance in the increase of microbial population of the soil, and consequently higher corn yield. Further studies with *Bacillus* sp., are still needed to determined its role as cell protector that keep viable during the seed germination period and others variables that can interfere with their survival. Nonetheless, there is a great potential for the development of commercial inoculants.

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Cited literature

Araújo, F. F. 2008. Inoculação de sementes com *Bacillus subtilis*, formulado com farinha de ostras e desenvolvimento de milho, soja e algodão. Ciência e Agrotecnologia. 2(32):456-462. https://doi.org/10.1590/S1413-70542008000200017.

Araújo, F. F. and Guerreiro, R. T. 2010. Bioprospection of *Bacillus* isolates promoters of corn growth in natural and sterile soil. Ciência e Agrotecnologia. 4(34):837-844. https://doi.org/10.1590/S1413-70542010000400007.

Buchelt, A. C.; Metzler, C. R.; Castiglioni, J. L.; Dassoller, T. F. y Lubian, M. S. 2019. Aplicação de bioestimulantes e *Bacillus subtilis* na germinação e desenvolvimento inicial da cultura do milho. Revista de Agricultura Neotropical. 4(6):69-74. https://doi.org/10.32404/rean.v6i4.2762.

Chagas, L. F. B.; Martins, A. L. L.; Carvalho F. M. R.; Miller, L. O.; Oliveira, J. C. y Chagas Junior, A. F. 2017. *Bacillus subtilis* e *Trichoderma* sp. no incremento da biomassa em plantas de soja, feijão-caupi, milho e arroz. Revista Agri-Environmental Sciences. 2(3):1-18. https://revista.unitins.br/index.php/agri-environmental-sciences/index.

CONAB. 2020. Companhia Nacional de Abastecimento. Acompanhamento da safra de grãos Safra 2019/2020. Brasil. 7(10):1-69.

Cunha, E. Q.; Stone, L. F.; Moreira, J. A. A.; Ferreira, E. P. B.; Didonet, A. D. y Leandro, W. M. 2011. Sistemas de preparo do solo e culturas de cobertura na produção orgânica de feijão e milho: I-Atributos físicos do solo. Rev. Brasileira de Ciência do Solo. 2(35):589-602. http://dx.doi.org/10.1590/S0100-06832011000200029.

Domenech, J.; Reddy, M. S.; Kloepper, J. W.; Ramos, B. and Gutierrez-Mañerro, J. 2006. Combined application of the biological product LS213 with *Bacillus*, *Pseudomonas* or *Chryseobacterium* for growth promotion and biological control of soil-borne diseases in pepper and tomato. BioControl. 51:245-258. Doi: 10.1007/s10526-005-2940-z.

Dotto, A. P.; Lana, M. C.; Steiner, F. y Frandoloso, J. F. 2010. Produtividade do milho em resposta à inoculação com *Herbaspirillum seropedicae* sob diferentes níveis de nitrogênio. Revista Brasileira de Ciências Agrárias. 3(5):376-382. Doi: 10.5039/agraria.v5i3a898.

EMBRAPA. 2006. Empresa Brasileira de Pesquisa Agropecuária. Centro Nacional de Pesquisa de Solos (Rio de Janeiro, RJ). Sistema brasileiro de classificação de solos. 2. (Ed.). Rio de Janeiro. EMBRAPA-SPI. 306 p.

Ferreira, D. F. 2019. SISVAR: a computer analysis system to fixed effects split plot type designs. Revista Brasileira de Biometria [S.l.]. 4(37):529-535. https://doi.org/10.28951/rbb.v37i4.450.

Ferreira, N. C.; Mazzuchelli, R. C. L.; Pacheco, A. C.; Araújo, F. F.; Antunes, J. E. L. y Araújo, A. S. F. 2018. *Bacillus subtilis* improves maize tolerance to salinity. Ciência Rural. 8(48):1-4. http://dx.doi.org/10.1590/0103-8478cr20170910.

Gama-Rodrigues E. F.; Barros, N. F.; Viana, A. P. y Santos, G. A. 2008. Alterações na biomassa e na atividade microbiana da serapilheira e do solo, em decorrência da substituição de cobertura florestal nativa por plantações de eucalipto, em diferentes sítios da região sudeste do Brasil. Revista Brasileira de Ciência do Solo. 4(32):1489-1499. http://dx.doi.org/10.1590/S0100-06832008000400013.

Hernández-Hernández, E. J.; Hernández-Rios, I.; Almaraz-Suarez, J. J.; López-López, A.; Torres-Aquino, M. y Flores, F. J. M. 2018. Caracterización *in vitro* de rizobacterias y su antagonismo com hongos causantes del damping off em Chile. Rev. Mex. Cienc. Agríc. 3(9):525-537. https://doi.org/10.29312/remexca.v9i3.335.

Jakelaitis, A.; Silva, A. A.; Santos, J. B. y Vivian, R. 2008. Qualidade da camada superficial de solo sob mata, pastagens e áreas cultivadas. Pesquisa Agropecuária Tropical. 2(38):118-127.

Lanna Filho, R.; Ferro, H. M. y Pinho, R. S. C. 2010. Controle biológico mediado por *Bacillus subtilis*. Revista Trópica: Ciências Agrárias e Biológicas. 2(4):12-20. http://dx.doi.org/10.0000/rtcab.v4i2.145.

Lazarovits, G. and Nowak, J. 1997. Rhizobacteria for improvement of plant growth and establishment. HortScience. 2(32):188-192. https://doi.org/10.21273/HORTSCI.32.2.188.

Lima, F.; Nunes, L. A. P. L.; Figueiredo, M. V. B.; Araújo, F. F.; Lima, L. M. y Araújo, A. S. F. 2011. *Bacillus subtilis* e adubação nitrogenada na produtividade do milho. Revista Brasileira de Ciência Agrárias. 4(6):657-661. Doi: 10.5039/agraria.v6i4a1429.

Machado, A. P. y Costa, M. J. N. 2018. Biocontrole do fitonematóide *Pratylenchus brachyurus* in vitro e na soja em casa de vegetação por *Bacillus subtilis*. Revista Biociências. 1(23):83-84.

Mariano, R. L. R.; Silveira, E. B.; Assis, S. M. P.; Gomes, A. M. A.; Nascimento, A. R. P. y Donato, V. M. T. S. 2004. Importância de bactérias promotoras de crescimento e de biocontrole de doenças de plantas para uma agricultura sustentável. Anais da Academia Pernambucana de Ciências Agronômica, Recife. 1(1):89-111.

Migliorini, P.; Lazarotto, M.; Müller, J.; Oruoski, P.; Bovolini, M. P.; Barbieri, M.; Tunes, L. V. M. y Muniz, M. F. B. Qualidade fisiológica sanitária e transmissão de patógenos em sementes de canola. Colloquium Agrariae. 3(13):67-76. Doi: 10.5747/ca.2017.v13.n3.a175.

Modolo, A. J.; Trogello, E.; Nunes, A. L.; Silveira, J. C. M. y Kolling, E. M. 2011. Efeito da compactação do solo sobre a semente no desenvolvimento da cultura do feijão. Acta Scientiarum Agronomy. 1(33):89-95. Doi: 10.4025/actasciagron.v33i1.4236.

Moreira, J. C. F. 2014. Milho safra submetido à inoculação com bactérias diazotróficas associativas e doses de nitrogênio. Dissertação de Mestrado, Universidade Federal do Mato Grosso, Rondonópolis, MT. 64 p.

Persello-Cartieaux, F.; Nussaume, L. and Robaglia, C. 2003. Tales from the undergroud: molecular plant-rhizobacteria interactions. Plant, Cell and Environment. 2(26):189-199. https://doi.org/10.1046/j.1365-3040.2003.00956.x.

Ratz, R. J.; Palácio, S. M.; Espinoza-Quiñones, F. R.; Vicentino, R. C.; Michelim, H. J. y Richter, L. M. 2017. Potencial biotecnológico de rizobactérias promotoras de crescimento de plantas no cultivo de milho e soja. Engevista. 4(19):890-905. Doi: 10.22409/engevista.v19i4.894.

Rodrigues, L. S.; Baldani, V. L. D.; Reis, V. M. y Baldani, J. I. 2006. Diversidade de bactérias diazotróficas endofíticas dos gêneros *Herbaspirillum* e *Burkholderia* na cultura do arroz inundado. Pesquisa Agropecuária Brasileira. 2(41):275-284. http://dx.doi.org/10.1590/S0100-204X2006000200012.

Shafi, J.; Tian, H. and Mingshan, Ji. 2017. *Bacillus* species as versatile weapons for plant pathogens: a review. Biotechnology & Biotechnological Equipment, Abingdon. 3(31):446-459. https://doi.org/10.1080/13102818.2017.1286950.

Silva, R. R. D.; Silva, M. L. N.; Cardoso, E. L.; Moreira, F. M. D. S.; Curi, N. y Alovisi, A. M. T. 2010. Biomassa e atividade microbiana em solo sob diferentes sistemas de manejo na região fisiográfica Campos das Vertentes-MG. Revista Brasileira de Ciência do Solo, 5(34):1584-1592. http://dx.doi.org/10.1590/S0100-06832010000500011.

Simão, G., Demétrio, G. B.; Paula, G. F.; Ladeira, D. C. y Matsumoto, L. S. 2020. Influence of spent coffee grounds on soil microbiological attributes and maize crop. Research, Society and Development. 8(9):1-16. Doi: https://doi.org/10.33448/rsd-v9i8.6400.

Stamford, N. P.; Stamford, T. L. M.; Andrade, D. E. G. T. y Michereff, S. J. 2005. Microbiota dos solos tropicais. *In*: ecologia e manejo de patógenos radiculares em solos tropicais. Michereff, S. J. Andrade, D. E. G. T. y Menezes, M. (Ed.). Imprensa Universitária, Recife: UFRPE. 61-92 pp.

Szilagyi-Zecchin, V. J.; Mógor, A. F.; Ruaro, L. y Röder, C. 2015. Crescimento de mudas de tomateiro (*Solanum lycopersicum*) estimulado pela bactéria *Bacillus amyloliquefaciens* subsp. plantarum FZB42 em cultura orgânica. Revista de Ciências Agrárias. 1(38):26-33.

Vance, E. D.; Brookes, P. C. and Jenkinson, D. S. 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry. 6(19):703-707. https://doi.org/10.1016/0038-0717(87)90052-6.

Venzke-Filho, S. P.; Feigl, B. J.; Piccolo, M. C.; Siqueira Neto, M. y Cerri, C. C. 2008. Biomassa microbiana do solo em sistema de plantio direto na região de Campos Gerais-Tibagi, PR. Revista Brasileira de Ciência do Solo. 2(32):599-610. https://doi.org/10.1590/S0100-06832008000200015.

Walkley, A. and Black, I. A. 1934. An examination of the degtjareff method for determining soil organic matter, and proposed modification of the chromic acid titration method. Soil Science. 1(37):29-38.

Wetler-Tonini, R. M. C.; Rezende, C. E. y Grativol, A. D. 2010. Degradação e biorremediação de compostos do petróleo por bactérias: revisão. Oecologia Australis. 4(14):1010-1020. Doi: 10.4257/oeco.2010.1404.11.