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Original Article

Relations between soil attributes and the abundance of *Bacillus thurigiensis* in the Cerrado of Maranhão state, Brazil

Relações entre atributos do solo e a abundância do *Bacillus thurigiensis* no Cerrado do estado do Maranhão, Brasil

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Abstract

The influence of abiotic factors on the abundance of microorganism populations in soil has been sparsely studied, especially regarding *Bacillus thuringiensis* (Bt) bacteria. Therefore, this research was aimed at analyzing the relationship between the chemical and textural characteristics of the soil of the Cerrado (savanna) of Maranhão State on the abundance of *Bacillus thuringiensis*. Soil samples were collected in different municipalities in eastern Maranhão: São Mateus do Maranhão, Alto Alegre, Coroatá, Timbiras and Codó. The soil samples were obtained in the 0-0.1 m layer for soil fertility and texture analysis. Then, in the same area for the isolation of Bt, 1 g of soil was collected. The colonies obtained in the isolation that featured morphological characteristics of *Bacillus* spp. were visualized under phase contrast microscopy. Principal component analysis, clustering and correlations were peformed. Results: The sand content correlated positively with the *Bacillus thuringiensis* index (iBt). The cluster analysis allowed for verifying that the soils not showed iBt in function of high concentrations of aluminum (Al) and potential acidity (H+Al). Considering as these attributes (Al and H+Al) alter the availability of P in the soil, the abundance of *Bacillus thuringiensis* may have been impaired by the deficiency of this element in the environment. Conclusion: Bt has correlations with soil texture, and high concentrations of aluminum and potential acidity in the soil influencing the permanence of *Bacillus thuringiensis* in Maranhão eastern Cerrado.

Keywords: bacteria, soil fertility, acidity.

Resumo

A influência de fatores abióticos sobre a abundância de populações de microrganismos no solo tem sido pouco estudada, principalmente com relação à bactéria *Bacillus thuringiensis (Bt)*. Assim, objetivou-se analisar a relação entre as características químicas e texturais do solo do Cerrado maranhense na abundância de *Bacillus thuringiensis*. As coletas de solo foram realizadas em municípios do leste maranhense: São Mateus do Maranhão, Alto Alegre, Coroatá, Timbiras e Codó. As amostras de solo foram obtidas na camada de 0-0.1 m, para análise da fertilidade do solo e sua textura. As colônias obtidas no isolamento que apresentaram características morfológicas de *Bacillus* spp. foram visualizadas sob microscopia de contraste de fase. Em seguida, na mesma área para o isolamento do Bt, foi coletado 1g de solo. De posse dos dados procedeu-se análise de componentes principais, agrupamentos e correlações. Resultados: A areia correlacionou-se positivamente com o índice de *Bacillus thuringiensis* (iBt). A análise de agrupamento permitiu verificar que os solos que não apresentaram iBt, possuíam altas concentrações de alumínio e acidez potencial (H+Al). Como estes atributos alteram a disponibilidade de P no solo, a abundância de *Bacillus thuringiensis* pode ter sido prejudicada pela deficiência deste elemento no ambiente. Conclusão: Bt possui correlações com a textura do solo, e altas concentrações de alumínio e acidez potencial no solo influenciam na permanência de *Bacillus thuringiensis* no Cerrado do leste maranhense.

Palavras-chave: bactéria, fertilidade do solo, acidez.

1. Introduction

Bacillus thuringiensis (Bt) is a ubiquitous, gram-positive, spore-forming bacterium, which has one or more insecticidal proteins used to control insect pests of the orders Lepidoptera, Diptera, and Coleoptera (Jouzani et al.,

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2017; Rabinovitch et al., 2017). Early research on the ecology of this bacterium worked with the hypothesis that *Bacillus thuringiensis* had its natural habitat within certain insect species (Prasertphon et al., 1973; Suzuki et al., 2004).

Soil is still noted today as one of the environments in which there is the greatest amount of Bt spores (Dagga et al., 2016; Khodyrev et al., 2020). This environment has the ability to provide microhabitats that vary in their nutrient availability, in their physicochemical characteristics, in the characteristics of soil aggregates (Moreira and Siqueira, 2006), in intraspecific, interspecific interactions, and among the abiotic factors of the external environment and the soil itself (Cotta, 2016; Fierer, 2017; Rahman et al., 2024).

The classic article by Bernhard et al. (1997) that analyzed 2363 soils from 80 countries showed the local and world variation in the population density of Bt. Bt is known to easily adapt to the conditions of a number of different soils (Mishra et al., 2017), which can only support a minimal amount for each bacterial population due to the limited nutritional resources that each microhabitat provides.

However, the influence of these factors on the abundance of soil microorganism populations is poorly known, in part because most microbes and their interactions cannot be directly observed or measured (Karimi et al., 2019). However, new *Bacillus thuringiensis* strains with high toxicity rate to *A. aegypti* have been found in Atlantic Forest soils (Santos et al., 2012). Similarly, other authors have collected them in Cerrado, Amazon and Caatinga soils (Soares-da-Silva et al., 2015), in restinga and mangrove soils (Vieira-Neta et al., 2021) and continue to raise questions about the characteristics and the influence of abiotic factors of the environment and the soil in obtaining *Bacillus thuringiensis* isolates.

Thus, the objective was to analyze the relationship between the chemical and textural characteristics of soil from areas of native Cerrado forest on the abundance of *Bacillus thuringiensis.*

2. Materials and Methods

2.1. Collection of soil for analysis of fertility, soil texture and Bacillus thuringiensis

In five municipalities from central to eastern Maranhão State, São Mateus do Maranhão (SM), Alto Alegre do Maranhão (AT), Coroatá (CT), Timbiras (TB) and Codó (CD), areas of the Cerrado biome (native forest) were selected, where data collection took place in January 2020 (Figure 1). The main soils in these municipalities are: (i) Ultisols (Argissolos), Oxisols (Latossolos) and Plinthic of Oxisols (Plintossolos) in São Mateus do Maranhão, (ii) Plinthic of Oxisols (Latossolos) and Ultisols (Argissolos) in Alto Alegre do Maranhã, (iii) Ultisols (Argissolos), Oxisols (Latossolos) and Plinthic of Oxisols (Plintossolos) in Coroatá, (iv) Ultisols (Argissolos), Oxisols (Latossolos) and Plinthic of Oxisols (Plintossolo) in Timbiras, and (v) Ultisols (Argissolo), Plinthic of Oxisols (Plintossolo) and Entisoils (Neossolo Quartzarênico) in Codó (Jacomine, 1986).

The collections in the conserved environments (native forest area) were conducted by collecting 15 sub-samples,

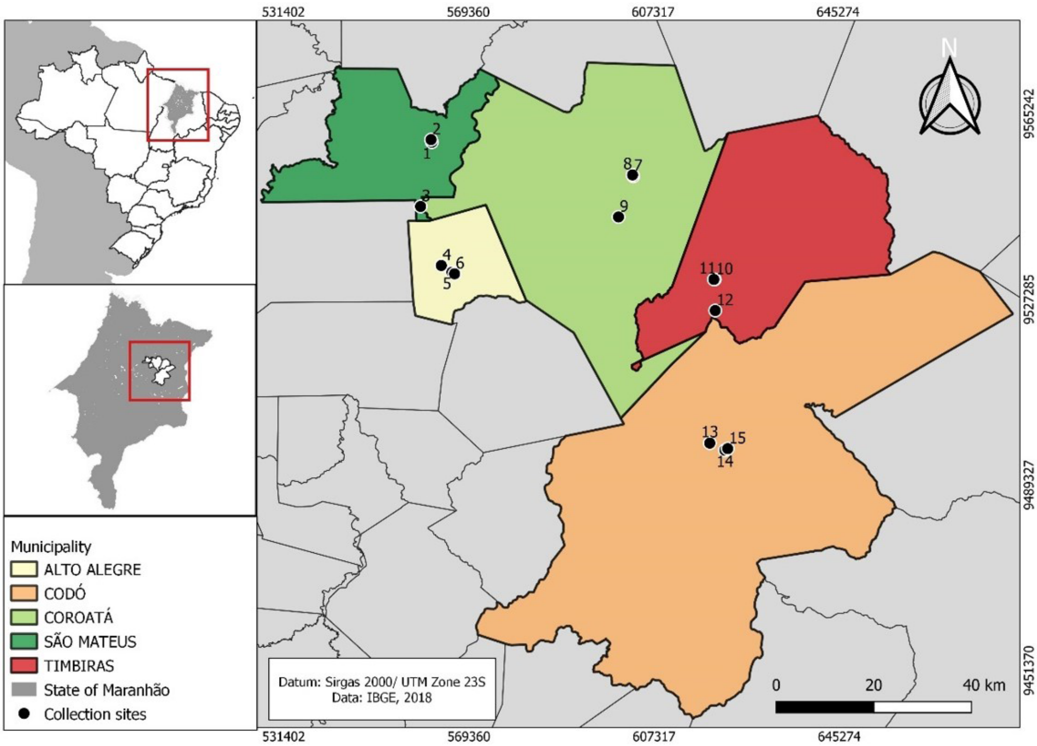

Figure 1. Soil sampling areas in the Cerrado, Maranhão state, Brazil.

randomly, and each sub-sample being 10 m apart, to form a composite sample in each area, three areas of native Cerrado forest per municipality being surveyed (sampled), totaling 15 total samples. The soil was collected using a Dutch auger, also used for the analysis of chemical and texture attributes, and specifically for the analysis of *Bacillus thuringiensis*; a wooden spatula was used for soil collection, with subsequent storage in previously sterilized Falcon-type centrifuge tubes.

2.2. Isolation of Bacillus thuringiensis

Isolation of *Bacillus thuringiensis* was performed in the Laboratory of Medical Entomology-LABEM - CESC/UEMA in compliance with the protocol recommended by the World Health Organization (WHO, 1985), which consisted of mixing 1g of soil from each sample to 10 ml of salt solution (0.006 mM $FeSO_4.7H_2O$; 0.01 mM $CaCO_3.7H_2O$; 0.08 mM $MgSO_4.7H_2O$; 0.07 mM $MnSO_4.7H_2O$; 0.006 mM $ZnSO_4.7H_2O$).

Next, the samples were then diluted serially (10^{-1} and 10^{-2}) in saline solution (NaCl at 1%). Subsequently, a 1ml aliquot of the last dilution was homogenized on a vortex tube beater and subjected to heat shock at 80°C for 12 min in a water bath, and then in ice for 5 min. After this process, 100 µl of the solution was transferred to Petri dishes containing nutrient agar (AN) culture medium (peptone 5 g/l, sodium chloride 5 g/l, meat extract 1.5 g/Ll yeast extract 1.5 g/l and agar 15 g/l) (Himedia). The dishes were inverted and stored in a bacteriological oven at 28 ºC for 48h for growth of bacterial colonies.

After growth, the colonies were evaluated for morphology (shape, edge, elevation, structure, size and color), according to Rampersad and Ammons (2005). Those colonies showing typical characteristics of *Bacillus* spp. were visualized by phase-contrast microscope (1000 X magnification) to check for the presence of paraspore inclusions (protein crystals). The colonies positive to *Bacillus* spp. were seeded on Petri dishes containing agar and incubated in a bacteriological incubator for 48 hours. Upon completion of this step, each colony was plated separately, incubated in an incubator for five days, and properly stored. For the Bt index (iBt), the percentage of colonies was calculated out of the total number of colonies obtained in the isolation.

The isolates identified as *Bacillus thuringiensis* were deposited in the Banco de Bacilos Entomapatogênicos do Maranhão - BBENMA (Entomapathogenic Bacilli Bank of Maranão State), at the Medical Entomology Laboratory of CESC/UEMA. For each isolate that was identified, a code formed by four letters (BtMA: Bt - Bacillus thuringiensis and MA - Maranhão) was generated, added by the number corresponding to its storage sequence in the Bank.

2.3. Analysis of chemical attributes and soil texture

The chemical attributes that were analyzed included: pH (H_2O), organic matter - MO (Walkley-Black method), phosphorus - P (method: Mehlich1 extractor), potassium - K^+ (method: Mehlich1 extractor), sodium - Na^+ (method: Mehlich1 extractor), calcium - Ca^{2+} (method: KCl extractor), magnesium - Mg^{2+} (method: KCl extractor), aluminum - Al^{3+} (method: KCl extractor), potential acidity - H+Al (method: extractor: Ca acetate), sum of bases (SB) = $K^++Ca^{2+}+Mg^{2+}+Na^+$, cation exchange capacity (CEC) = SB + H+Al, base saturation (BS) = (SB/CEC)*100 and aluminum saturation were also calculated (m) = Al/(SB+Al)*100; sand, silt and clay contents (pipette method) were also determined (Teixeira et al., 2017).

2.4. Statistical analysis

Once data from the soil fertility and texture analysis and the *Bacillus thuringiensis* colony index (iBt) were obtained, they were submitted to principal component analysis (PCA) (Jolliffe, 1986) and clustering using Euclidean distance and the UPGMA (Unweighted Pair-Group Average) method. The analyses were performed by the R statistical computing software (R Development Core Team, 2020) using the packages "FactoMineR" (Lê et al., 2008) e "factoextra" (Kassambara and Mundt, 2020).

3. Results

The 15 soils collected permitted obtained 192 bacterial colonies and 63 (32.8%) were identified as *Bacillus thuringiensis*. The rate of *Bacillus thuringiensis* colonies (iBt) in relation to the number of bacterial colonies varied from zero to 0.64 (Table 1).

The value mean of attributes of soil fertility were 4.42 for pH, 0.9 (dag kg^{-1}) for OM, 6.49 (mg dm^{-3}) for P, 0.25 ($cmol_c$ dm^{-3}) for K, 0.05 ($cmol_c$ dm^{-3}) for Na, 2.12 ($cmol_c$ dm^{-3}) for Ca, 0.91 ($cmol_c$ dm^{-3}) for Mg, 1.54 ($cmol_c$ dm^{-3}) for Al, 10.44 ($cmol_c$ dm^{-3}) for H+Al, 3.32 ($cmol_c$ dm^{-3}) for SB, 13.76 ($cmol_c$ dm^{-3}) for CEC, 23.57 (%) for BS, 32.67 (%) for m and the mean content of sand, clay and silt were 56.92, 13.80 and 29.28 (%), respectively.

Despite being considered areas of native vegetation, the areas under analysis show significant variability for chemical attributes and soil texture, the coefficient of variation presented the following decreasing order for the analyzed attributes: Na > Al > K > m > Mg > Clay > Ca > SB > Silt > BS > Sand > H+Al > CEC > OM > P > pH (Table 1).

Principal component analysis generated three components that explained 87.04% of the isolation and attribute mean data (Table 2). In the PCA, the Cos2 and the contributions (%) were used to select the variables with the greatest contribution to the variability of the data. The first two components explained 76.18% of the variability of the samples. The first principal component correlated with twelve of the 17 variables studied. The attributes K, Ca, Mg, H+Al, OM, SB, CEC, BS, clay and silt correlated highly positively and negatively with sand and iBt (Table 3 and Figure 2).

In the cluster analysis of the similarity between the different sampled points, three groups were formed: (G1) composed of samples SM01, SM02, SM03, TB11, CD13, CD14 and CD15; (G2) composed of samples CT07 and CT08, and (G3) composed of samples AT04, AT05, AT06, CT09, TB10 and TB12 (Figure 3). And Table 4 shows the means of chemical and physical attributes of three groups formed, G1 – iBt: 0.46 (%), pH: 4.26, OM: 0.75 (dag kg^{-1}), P: 6.75 (mg dm^{-3}), K: 0.12 ($cmol_c$ dm^{-3}), Na: 0.04 ($cmol_c$ dm^{-3}),

Table 1. Values of bacterial colony number, Bt, iBt, chemical and physical attributes in soil samples collected in different municipalities in the Maranhão eastern Cerrado.

Sample	No. of bacterial colonies	No. of Bt colonies	iBt	pH	OM	P	K	Na	Ca	Mg	Al	H+Al	SB	CEC	BS	m	Sand	Clay	Silt
	(ncb)	(nbt)	%	(H_2O)	(dag kg^{-1})	(mg dm^{-3})	(cmol$_c$ dm^{-3})								(%)				
SM01	14	4	0.29	4.24	0.52	6.39	0.11	0.11	1.29	0.42	2.38	8.89	1.93	10.82	17.84	55.22	56.10	10.29	33.61
SM02	8	5	0.63	4.2	0.83	6.64	0.06	0.02	0.98	0.39	1.7	7.55	1.44	8.99	16.04	54.14	79.50	7.22	13.28
SM03	10	3	0.3	4.05	1.03	5.41	0.06	0.02	1.47	0.48	2.99	10.83	2.02	12.85	15.69	59.71	74.15	5.98	19.87
AT04	5	1	0.2	4.84	0.61	4.59	0.14	0.02	2.44	0.48	0.14	6.5	3.08	9.59	32.14	4.35	61.31	6.95	31.74
AT05	15	5	0.33	4.82	0.94	3.93	0.4	0.03	3.26	1.42	0.19	9.82	5.11	14.93	34.21	3.53	57.45	12.57	29.98
AT06	10	1	0.1	4.4	0.85	6.8	0.4	0.03	2.12	1.4	0.7	9.35	3.94	13.29	29.65	15.09	49.55	12.69	37.77
CT07	17	0	0	4.35	1.48	6.23	0.28	0.03	3.94	1.1	1.84	15.18	5.35	20.53	26.05	25.64	21.51	27.92	50.57
CT08	11	0	0	4.18	1.34	3.19	0.45	0.05	2.77	1.94	4.74	18.91	5.21	24.13	21.6	47.61	27.37	27.61	45.02
CT09	16	6	0.38	4.92	0.76	10.49	0.31	0.03	3.82	1.09	0.07	7.68	5.25	12.94	40.61	1.31	58.71	10.31	30.98
TB10	11	7	0.64	4.39	1.21	10.08	0.57	0.14	2.88	0.95	1.17	13.07	4.53	17.6	25.76	20.46	51.98	18.14	29.88
TB11	20	10	0.5	4.25	0.89	7.54	0.34	0.11	1.13	0.71	2.19	12.79	2.28	15.07	15.15	48.99	48.91	20.54	30.55
TB12	20	3	0.15	4.59	0.99	4.83	0.34	0.05	2.62	1.79	1.12	10.48	4.79	15.28	31.38	18.94	36.89	20.04	43.07
CD13	18	11	0.61	4.42	0.71	6.31	0.17	0.02	1.26	0.73	1.21	10.7	2.18	12.88	16.91	35.77	61.43	11.9	26.67
CD14	10	5	0.5	4.27	0.77	7.95	0.06	0	1.03	0.34	1.47	8.03	1.42	9.45	15.05	50.81	82.81	7.71	9.48
CD15	7	3	0.43	4.37	0.52	7.04	0.06	0.02	0.83	0.34	1.17	6.77	1.24	8.01	15.49	48.47	86.15	7.19	6.66
Total	192	64	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean	12.8	4.27	0.34	4.42	0.90	6.49	0.25	0.05	2.12	0.91	1.54	10.44	3.32	13.76	23.57	32.67	56.92	13.80	29.28
CV(%)	36.90	77.46	64.19	5.90	31.34	31.24	66.81	90.52	50.11	59.10	79.22	32.55	48.44	32.30	35.76	63.94	33.35	53.24	43.29

iBt: index of *Bacillus thuringiensis*; OM: organic matter; P: phosphorus; K: potassium; Na: sodium; Ca: calcium; Mg: magnesium; Al: aluminum; H+Al: potencial acidity; SB: sum of bases; CEC: cation exchange capacity; BS: base saturation; m: aluminum saturation; SM: São Mateus do Maranhão; AT: Alto Alegre; CT: Coroatá; TB: Timbiras; CD: Codó.

Table 2. Estimated variances (eigenvalues) and accumulated percentage of the total variance (%) obtained through the principal component (PC) analysis considering 15 samples of soils in the Maranhão eastern Cerrado.

PC	Eigenvalues	% Accumulated
1	8.63	50.74
2	4.33	76.18
3	1.85	87.04

Table 3. Correlation, quality of representation (Cos2) and contribution (%) between original variables and principal components (PC) in iBt and the chemical and physical attributes of soils in the Maranhão eastern Cerrado.

Variables	Correlation		Cos2	Contribution (%)
	PC 1	PC2		
iBt	-0.64**	-0.04	0.41	3.17
pH	0.23	-0.93**	0.92	7.08
OM	0.77**	0.38	0.73	5.67
P	-0.24	-0.25	0.12	0.93
K	0.83**	-0.10	0.70	5.37
Na	0.29	0.23	0.14	1.04
Ca	0.83**	-0.44	0.87	6.73
Mg	0.88**	-0.06	0.77	5.98
Al	0.18	0.92**	0.88	6.82
H+Al	0.75**	0.62*	0.95	7.30
SB	0.93**	-0.31	0.97	7.47
CEC	0.91*	0.36	0.96	7.40
BS	0.57*	-0.80**	0.96	7.43
m	-0.49	0.86**	0.97	7.48
Sand	-0.94**	-0.15	0.90	6.98
Clay	0.86**	0.38	0.88	6.78
Silt	0.91**	0.01	0.82	6.37

**Significant at 1%; *Significant at 5%. iBt: index of *Bacillus thuringiensis*; OM: organic matter; P: phosphorus; K: potassium; Na: sodium; Ca: calcium; Mg: magnesium; Al: aluminum; H+Al: potencial acidity; SB: sum of bases; CEC: cation exchange capacity; BS: base saturation; m: aluminum saturation.

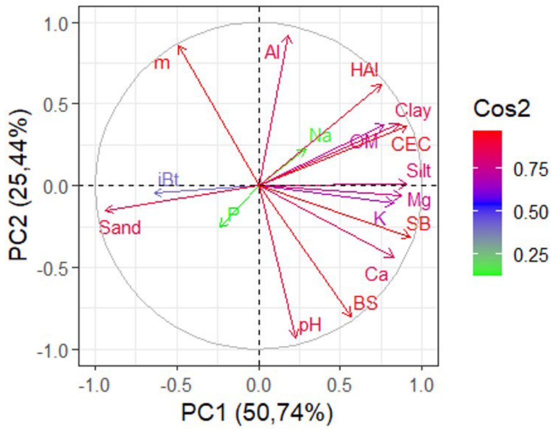

Figure 2. Biplot showing the association between iBt, chemical and physical attributes in soil samples in the Maranhão eastern Cerrado. Note: High Cos2 values are associated with a color scale and proximity to the circle of correlations; the warmer the color (red) and closer to the circle of correlation the greater the importance of these variables for the interpretation of these components. iBt: index of *Bacillus thuringiensis*; OM: organic matter; P: phosphorus; K: potassium; Na: sodium; Ca: calcium; Mg: magnesium; Al: aluminum; H+Al: potencial acidity; SB: sum of bases; CEC: cation exchange capacity; BS: base saturation; m: aluminum saturation.

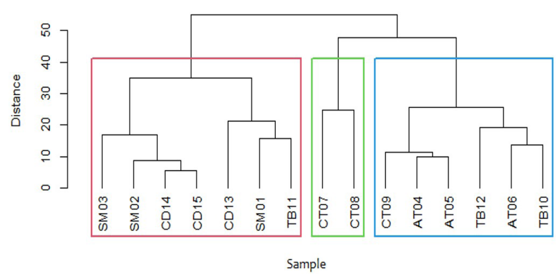

Figure 3. Similarity dendrogram between iBt, chemical and physical soil attributes in the Maranhão eastern Cerrado. Notes: A: Group 1 (G1) in the red line cluster, Group 2 (G2) in the green line cluster, Group 3 (G3) in the purple line cluster. B: Legend of the abbreviations SM: São Mateus do Maranhão; AT: Alto Alegre; CT: Coroatá; TB: Timbiras; CD: Codó. Source: Authors.

Ca: 1.14 (cmol$_c$ dm^{-3}), 0.49 (cmol$_c$ dm^{-3}), Al: 1.87 (cmol$_c$ dm^{-3}), H+Al: 9.37 (cmol$_c$ dm^{-3}), SB: 1,79 (cmol$_c$ dm^{-3}), CEC: 11.15 (cmol$_c$ dm^{-3}), BS: 16.03 (%), m: 50.44 (%), Sand: 69.86 (%), Clay: 10.12 (%) and Sil: 20.02 (%); G2 – iBt: 0.00 (%), pH: 4,27, OM: 1.41 (dag kg^{-1}), P: 4.71 (mg dm^{-3}), K: 0.37 (cmol$_c$ dm^{-3}), Na: 0.04 (cmol$_c$ dm^{-3}), Ca: 3.35 (cmol$_c$ dm^{-3}), Mg: 1.52 (cmol$_c$ dm^{-3}), Al: 3.29 (cmol$_c$ dm^{-3}), H+Al: 17.05 (cmol$_c$ dm^{-3}), SB: 5.28 (cmol$_c$ dm^{-3}), CEC: 22.33 (cmol$_c$ dm^{-3}), BS: 23.83 (%), m: 36.62 (%), Sand: 24.44 (%), Clay: 27.77 (%) and Silt: 47.80 (%); and G3 – iBt: 0.30 (%), pH: 4.66, OM: 0.89 (dag kg^{-1}), P: 6.70 (mg dm^{-3}), K: 0.36 (cmol$_c$ dm^{-3}), Na: 0.05 (cmol$_c$ dm^{-3}), Ca: 2.86 (cmol$_c$ dm^{-3}), Mg: 1.19 (cmol$_c$ dm^{-3}), Al: 0.56 (cmol$_c$ dm^{-3}), H+Al: 9.49 (cmol$_c$ dm^{-3}), SB: 4.45 (cmol$_c$ dm^{-3}), CEC: 13.94 (cmol$_c$ dm^{-3}), BS: 32.29 (%), m: 10.61 (%), Sand: 52.65 (%), Clay: 13.45 (%) and Silt: 33.90 (%).

4. Discussion

The soils from these areas feature a sandy texture, with low organic matter values and base saturation, and high aluminum concentrations (Sousa et al., 2004; Donagemma et al., 2016).

The results obtained in this research agree with the work of Lobo et al. (2018), in the Cerrado in Maranhão State, the percentage found in 45 samples was 31.2% of Bt. It is known that access to substrate and energy sources vary among microhabitats (Moreira and Siqueira, 2006). In microhabitat,

Table 4. Mean values of iBt, chemical and physical attributes in soil samples, collected in the Maranhão eastern Cerrado for different sample groups.

Groups	iBt	pH	OM	P	K	Na	Ca	Mg	Al	H+Al	SB	CEC	BS	m	Sand	Clay	Silt
	%	(H_2O)	(dag kg^{-1})	(mg dm^{-3})	($cmol_c$ dm^{-3})								(%)				
G1	0.46	4.26	0.75	6.75	0.12	0.04	1.14	0.49	1.87	9.37	1.79	11.15	16.03	50.44	69.86	10.12	20.02
G2	0.00	4.27	1.41	4.71	0.37	0.04	3.35	1.52	3.29	17.05	5.28	22.33	23.83	36.62	24.44	27.77	47.80
G3	0.30	4.66	0.89	6.79	0.36	0.05	2.86	1.19	0.56	9.49	4.45	13.94	32.29	10.61	52.65	13.45	33.90

G1: SM01, SM02, SM03, TB11, CD13, CD14 and CD15; G2: CT07 and CT08; G3: AT04, AT05, AT06, TB10, TB12 and CT09. iBt: index of *Bacillus thuringiensis*; OM: organic matter; P: phosphorus; K: potassium; Na: sodium; Ca: calcium; Mg: magnesium; Al: aluminum; H+Al: potencial acidity; SB: sum of bases; CEC: cation exchange capacity; BS: base saturation; m: aluminum saturation.

however, the percentages found in 1g of Cerrado soil may point to a minimum amount of environment support for the survival of *Bacillus thuringiensis* per gram of soil.

The variation obtained in the index presented therefore enhancing the fact that in each microenvironment there is competition for nutrients, beneficial interactions, and antagonistic inhibitions that can make microbial abundance restricted or enhanced (Karimi et al., 2019).

The biochemistry of the soil solution is mainly formed by acid-base and redox reactions (Voroney and Heck, 2015). These reactions can affect the microorganisms in their enzymatic functions, or can favor one or another antagonist (Voroney and Heck, 2015). In the analyzed soil, which is acidic and of low fertility, these results point to the metabolic adaptability of *Bacillus thuringensis*, confirming its characteristic of generalist bacteria and its presence in various types of soil.

Considering that the soils evaluated are native Cerrado, whose characteristic is low fertility, with high values of attributes linked to acidity and aluminum, and low in basic cations and phosphorus (Amorim et al., 2020), thus we can justify the contrasting result between the mentioned chemical attributes and iBt, since there is a need for an adequate pH and nutrient concentrations for a satisfactory development of microorganisms (Medhi et al., 2017), including Bt, as reported in Rabinovitch et al. (2017). Furthermore, for a good conditions for bacterial growth includ temperature and pH, so in low value of pH, that represent high levels of H+Al and Al in soils of Cerrado could be explain the results founded (Kalsoom et al., 2023).

As for the positive relationship between iBt and sand content shown in the multivariate analysis, this may be linked to better aeration and mineral stability that sand may favor *Bacillus thuringiensis*. Evidence of this association of Bt with sand content had already been pointed out by Hossain et al. (1997), in soils of Bangladesh, which indicates that the texture may exert some influence on the survival of *Bacillus thuringiensis* in the soil.

The contributions, BS and m were the variables that contributed most to the variability of the principal components (Table 4). In relation, the response variable, iBt, the m in the first component has a directly proportional relationship and inversely in the second component (Figure 2).

Thus, it can be seen that G2, composed of samples CT07 and CT08, presented zero iBt (Table 4) and are located in soils that have high concentrations of aluminum and potential acidity (H+Al). High values of Al and H+Al are characteristic of acidic soils, which act on P fixation by forming stable compounds and decreasing the availability of this nutrient, which makes it non-label for plants and microorganisms (Paul et al., 2017). Moreover, this group exhibits the highest values of clay, which is also a factor that contributes to greater P fixation (Zhang et al., 2019).

In a soil with lower phosphorus content, this becomes a selecting factor that can limit the amount of individuals of a microbial community as well as its establishment and development in local soils, besides being a limiting factor for rare microbial communities (Peng et al., 2021). Therefore, the lower availability of P may also have contributed to the absence of Bt in this cluster.

Phosphorus deficiency limits the reproduction and productivity of any microorganism, mainly due to its presence in DNA molecules. In addition, *Bacillus thuringiensis* is part of a group of bacteria that is able to solubilize unavailable phosphate to available phosphate, thus due to an absence of this attribute in the soil the metabolism of this bacterium can become inactive and thus contribute to the decline in population rate (Delfim et al., 2018).

5. Conclusion

There are a local variation in the *Bacillus thuringiensis* population associated with the soil texture of the Cerrado, with positive relationship between iBt and sand content.

The absence of *Bacillus thuringiensis* in soils of Maranhão's eastern Cerrado is associated with higher concentration of potential acidity (H+Al) and aluminum (Al).

The groups formed by cluster analysis permitted differing the characteristics that proportion the presence (high value of P and low value of Al, H+Al and clay) or absence (low values of P and high values of Al, H+Al and clay) of iBt.

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References

AMORIM, S.P.N., BOECHAT, C.L., DUARTE, L.S., ROCHA, C.B. and CARLOS, F.S., 2020. Grasses and legumes as cover crops affect microbial attributes in oxisol in the cerrado (savannah environment) in the northeast region. *Revista Caatinga*, vol. 33, no. 1, pp. 31-42. http://dx.doi.org/10.1590/1983-21252020v33n104rc.

BERNHARD, K., JARRETT, P., MEADOWS, M., BUTT, J., ELLIS, D.J., ROBERTS, G.M., PAULI, S., RODGERS, P. and BURGES, H.D., 1997. Natural isolates of *Bacillus thurigiensis*: worldwide distribution, characterization, and activity against insect pests. *Journal of Invertebrate Pathology*, vol. 70, no. 1, pp. 59-68. http://dx.doi.org/10.1006/jipa.1997.4669.

COTTA, S.R., 2016. O solo como ambiente para a vida microbriana. In: E.J.B.N. CARDOSO and F.D. ANDREOTE, eds. *Microbiologia do solo*. Piracicaba: Escola Superior de Agricultura Luiz Queiroz, pp. 26-36.

DAGGA, A., AZIZ, M.A., AMNAMA, A.A., AL-SHARIF, M. and HINDI, M., 2016. Isolation and molecular characterization of cry gene for *Bacillus thuringiensis* isolated from soil of Gaza Strip. *International Journal of Current Microbiology and Applied Sciences*, vol. 5, no. 4, pp. 659-666. http://dx.doi.org/10.20546/ijcmas.2016.504.075.

DELFIM, J., SCHOEBITZ, M., PAULINO, L., HIRZEL, J. and ZAGAL, E., 2018. Phosphorus availability in wheat, in volcanic soils inoculated with phosphate-solubilizing *Bacillus thuringiensis. Sustainability*, vol. 10, no. 2, p. 144. http://dx.doi.org/10.3390/su10010144.

DONAGEMMA, G.K., FREITAS, P.L., BALIEIRO, F.C., FONTANA, A., SPERA, S.T., LUMBRERAS, J.F., VIANA, J.H.M., ARAÚJO FILHO, J.C., SANTOS, F.C., ALBUQUERQUE, M.R., MACEDO, M.C.M., TEIXEIRA, P.C., AMARAL, A.J., BORTOLON, E. and BORTOLON, L., 2016. Characterization, agricultural potential, and perspectives for the management of light soils in Brazil. *Pesquisa Agropecuária Brasileira*, vol. 51, no. 9, pp. 1003-1020. http://dx.doi.org/10.1590/s0100-204x2016000900001.

FIERER, N., 2017. Embracing the unknown: disentangling the complexities of the soil microbiome. *Nature Reviews. Microbiology*, vol. 15, no. 10, pp. 579-590. http://dx.doi.org/10.1038/nrmicro.2017.87. PMid:28824177.

HOSSAIN, M.A., AHMED, S. and HOQUE, S., 1997. Abundance and distribution of Bacillus thuringiensis in the agricultural soil of Bangladesh. *Journal of Invertebrate Pathology*, vol. 70, no. 3, pp. 221-225. http://dx.doi.org/10.1006/jipa.1997.4694. PMid:9367730.

JACOMINE, P.K.T., 1986. *Levantamento exploratório-reconhecimento de solos do estado do Maranhão*. Rio de Janeiro: Embrapa Solos.

JOLLIFFE, I.T., 1986. *Principal component analysis*. New York: Springer. http://dx.doi.org/10.1007/978-1-4757-1904-8.

JOUZANI, G.S., VALIJANIAN, E. and SHARAFI, R., 2017. *Bacillus thuringiensis*: a successful insecticide with new environmental features and tiding. *Applied Microbiology and Biotechnology*, vol. 101, no. 7, pp. 2691-2711. http://dx.doi.org/10.1007/s00253-017-8175-y. PMid:28235989.

KALSOOM, BATOOL, A., DIN, G., DIN, S.U., JAMIL, J., HASAN, F., KHAN, S., BADSHAH, M. and SHAH, A.A., 2023. Isolation and screening of chromium resistant bacteria from industrial waste for bioremediation purposes. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 83, p. e242536. http://dx.doi.org/10.1590/1519-6984.242536. PMid:34495143.

KARIMI, B., DEQUIEDT, S., TERRAT, S., JOLIVET, C., ARROUAYS, D., WINCKER, P., CRUAUD, C., BISPO, A., PRÉVOST-BOURÉ, N.C. and RANJARD, L., 2019. Biogeography of soil bacterial networks along a gradient of cropping intensity. *Scientific Reports*, vol. 9, no. 1, p. 3812. http://dx.doi.org/10.1038/s41598-019-40422-y. PMid:30846759.

KASSAMBARA, A. and MUNDT, F., 2020 [viewed 23 June 2022]. *FactoExtra: extract and visualize the results of multivariate data analyses* [online]. R Development Core Team. Available from: https://cran.r-project.org/web/packages/factoextra/index.html

KHODYREV, V.P., DUBOVSKIY, I.M. and POLENOGOVA, O.V.A., 2020. New strain of *Bacillus thuringiensis* isolated from frozen soil of the Magadan region. *The Biological Bulletin*, vol. 47, no. 6, pp. 576-584. http://dx.doi.org/10.1134/S1062359020060072.

LÊ, S., JOSSE, J. and HUSSON, F., 2008. FactoMineR: an R package for multivariate analysis. *Journal of Statistical Software*, vol. 25, no. 1, pp. 1-18. http://dx.doi.org/10.18637/jss.v025.i01.

LOBO, K.S., SOARES-DA-SILVA, J., SILVA, M.A., TADEI, W.P., POLANCZYK, R.A. and PINHEIRO, V.C., 2018. Isolation and molecular characterization of *Bacillus thuringiensis* found in soils of the Cerrado region of Brazil, and their toxicity to *Aedes aegypti* larvae. *Revista Brasileira de Entomologia*, vol. 62, no. 1, pp. 5-12. http://dx.doi.org/10.1016/j.rbe.2017.11.004.

MEDHI, K., BHARDWAJ, R. and LAXMI, R., 2017. Climate change with its impacts on soil and soil microbiome regulating biogeochemical nutrient transformations. In: D.K. CHOUDHARY, A. MISHRA and A. VARMA, ed. *Climate change and the microbiome: sustenance of the ecosphere*. Cham: Springer, pp. 95-138. Soil Biology, no. 63.

MISHRA, P.K., BISHT, S.C., RUWARI, P., SUBBANNA, A.R.N.S., BISHT, J.K., BHATT, J.C. and GUPTA, H.S., 2017. Genetic diversity and functional characterization of endophytic *Bacillus thuringiensis* isolates from the north western Indian Himalayas. *Annals of Microbiology*, vol. 67, no. 2, pp. 143-155. http://dx.doi.org/10.1007/s13213-016-1244-0.

MOREIRA, F.M.S. and SIQUEIRA, J.O., 2006. *Microbiologia e bioquímica do solo*. Lavras: UFLA, 625 p.

PAUL, R., SINGH, R.D., PATRA, A.K., BISWAS, D.R., BHATTACHARYYA, R. and ARUNKUMAR, K., 2017. Phosphorus dynamics and solubilizing microorganisms in acid soils under different land uses of Lesser Himalayas of India. *Agroforestry Systems*, vol. 92, pp. 449-461. http://dx.doi.org/10.1007/s10457-017-0168-4.

PENG, Z., WANG, Z., LIU, Y., YANG, T., CHEN, W., WEI, G. and JIAO, S., 2021. Soil phosphorus determines the distinct assembly strategies for abundant and rare bacterial communities during successional reforestation. *Soil Ecology Letters*, vol. 3, no. 4, pp. 342-355. http://dx.doi.org/10.1007/s42832-021-0109-z.

PRASERTPHON, S., AREEKUL, P. and TANADA, Y., 1973. Sporulation of *Bacillus thuringiensis* in host cadavers. *Journal of Invertebrate Pathology*, vol. 21, no. 2, pp. 205-207. http://dx.doi.org/10.1016/0022-2011(73)90203-6.

R DEVELOPMENT CORE TEAM, 2020. *R: a language and environment for statistical computing* [software]. Vienna: R Foundation for Statistical Computing.

RABINOVITCH, L., VIVONI, A.M., MACHADO, V., KNAAK, N., BERLITZ, D.L., POLANCZYK, R.A. and FIUZA, L.M., 2017. *Bacillus thuringiensis* characterization: morphology, physiology, biochemistry, pathotype, cellular, and molecular aspects. In: L. FIUZA, R. POLANCZYK and N. CRICKMORE, eds. *Bacillus thuringiensis and Lysinibacillus sphaericus*. Cham: Springer, pp. 1-18. http://dx.doi.org/10.1007/978-3-319-56678-8_1.

RAHMAN, M., ZHANG, K., WANG, Y., AHMAD, B., AHMAD, A., ZHANG, Z., KHAN, D., MUHAMMAD, D. and ALI, A., 2024. Variations in soil physico-chemical properties, soil stocks, and soil stoichiometry under different soil layers, the major forest region Liupan mountains of northwest China. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 84, p. e256565. http://dx.doi.org/10.1590/1519-6984.256565. PMid:35195172.

RAMPERSAD, J. and AMMONS, D.A., 2005. Bacillus thuringiensis isolation method utilizing a novel stain, low selection and high throughput produced atypical results. *BMC Microbiology*, vol. 5, no. 1, p. 52. http://dx.doi.org/10.1186/1471-2180-5-52. PMid:16181492.

SANTOS, F.P., LOPES, J., VILAS-BÔAS, G.T. and ZEQUI, J.A.C., 2012. Characterization of *Bacillus thuringiensis* isolates with potential for control of *Aedes aegypti* (Linnaeus, 1762) (*Diptera*: culicidae). *Acta Tropica*, vol. 122, no. 1, pp. 64-70. http://dx.doi.org/10.1016/j.actatropica.2011.11.018. PMid:22178674.

SOARES-DA-SILVA, J., PINHEIRO, V.C.S., LITAIFF-ABREU, E., POLANCZYK, R.A. and TADEI, W.P., 2015. Isolation of *Bacillus thuringiensis* from the state of Amazonas, in Brazil, and screening against *Aedes aegypti* (Diptera, Culicidae). *Revista Brasileira de Entomologia*, vol. 59, no. 1, pp. 1-6. http://dx.doi.org/10.1016/j.rbe.2015.02.001.

SOUSA, D.M.G., LOBATO, E. and REIN, T.A., 2004. Adubação com fósforo. In: D.M.G SOUSA and E. LOBATO, eds. *Cerrado: correção do solo e adubação*. 2. ed. Planaltina: Embrapa Cerrados, pp. 147-168.

SUZUKI, M.T., LERECLUS, D. and ARANTES, O.M.N., 2004. Fate of *Bacillus thuringiensis* strains in different insect larvae. *Canadian*

Journal of Microbiology, vol. 50, no. 11, pp. 973-975. http://dx.doi.org/10.1139/w04-087. PMid:15644915.

TEIXEIRA, P.C., DONAGEMMA, G.K., FONTANA, A. and TEIXEIRA, W.G., 2017. *Manual de métodos de análise de solo*. 3rd ed. Brasília: Embrapa, 574 p.

VIEIRA-NETA, M.R.A., SOARES-DA-SILVA, J., VIANA, J.L., SILVA, M.C., TADEI, W.P. and PINHEIRO, V.C.S., 2021. Strain of *Bacillus thuringiensis* from Restinga, toxic to *Aede*s (*Stegomyia*) *aegypti* (Linnaeus) (Diptera, Culicidae). *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 81, no. 4, pp. 872-880. http://dx.doi.org/10.1590/1519-6984.228790. PMid:33053121.

VORONEY, R.P. and HECK, R.J., 2015. The soil habitat. In: E.A. PAUL, ed. *Soil microbiology, ecology an biochemistry*. 4th ed. Amsterdam: Elsevier, pp. 15-39. http://dx.doi.org/10.1016/B978-0-12-415955-6.00002-5.

WORLD HEALTH ORGANIZATION – WHO, 1985. *Informal consultation on the development of Bacillus sphaericus a microbial larvicide*. Geneva: WHO.

ZHANG, L., HU, Y., HAN, F., WU, Y., TIAN, D., SU, M., WANG, S., LI, Z. and HU, S., 2019. Influences of multiple clay minerals on the phosphorus transport driven by Aspergillus niger. *Applied Clay Science*, vol. 177, pp. 12-18. http://dx.doi.org/10.1016/j.clay.2019.04.026.