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Soil fungal community and chemical properties are not directly affected by Bt cotton

A comunidade fúngica e as propriedades químicas do solo não são diretamente afetadas pelo algodão Bt

La comunidad fúngica y las propiedades químicas del suelo no son afectadas directamente por el algodón Bt

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Abstract
Transgenic cotton has been cultivated in Brazil since 2005, including the plants which express the *cry* gene, derived from the entomopathogenic bacterium *Bacillus thuringiensis* (Bt). This technology has been considered efficient to control the target insect pests. However, little is known about whether these transgenic plants may impact the soil fungi and soil chemistry. This study aimed to evaluate the effects of genetically modified cotton resistant to insects on the soil fungal community and soil chemistry. The experiment was conducted in the agricultural area of Universidade Federal da Grande Dourados, Dourados Municipality, Mato Grosso do Sul State, Brazil. Two cotton (*Gossypium hirsutum* L.; Malvales: Malvaceae) cultivars were planted, one expressing a gene from Bt and the other without the foreign gene. Soil samples were collected monthly throughout the crop cycle. The amount of fungi in the soil was obtained by cultivation in Petri dishes, in triplicate, using Martin's culture medium and soil serial dilutions. From the isolates, the morphospecies were identified by amplification and sequencing of the Internal Transcribed Spacer (ITS) region of rDNA. The number of fungal colony-forming units and species richness were not directly influenced by the type of cotton grown or by the different cotton growing stages. Similarly, cultivation of Bt cotton did not affect soil chemistry, but differences were observed regarding different sampling times. Overall, our research contributes to the current knowledge regarding agriculture in tropical areas, showing that there is no apparent evidence of the effect of Bt cotton on soil fungal communities and chemistry.
Keywords: Transgenic cotton; Microbiota; Diversity; Soil ecology; Fungi.

Resumo
O algodão transgênico tem sido cultivado no Brasil desde 2005, incluindo plantas que expressam o gene *cry*, derivado da bactéria entomopatogênica *Bacillus thuringiensis* (Bt). Essa tecnologia tem sido considerada eficiente no controle de insetos-praga-alvo. No entanto, pouco se sabe sobre se essas plantas transgênicas podem impactar os fungos do

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solo e a química do solo. Este estudo teve como objetivo avaliar os efeitos do algodão geneticamente modificado resistente a insetos sobre a comunidade fúngica e composição química do solo. O experimento foi conduzido na área agrícola da Universidade Federal da Grande Dourados, município de Dourados, estado de Mato Grosso do Sul, Brasil. Duas cultivares de algodão (*Gossypium hirsutum* L.; Malvales: Malvaceae) foram plantadas, uma expressando um gene *Bt* e outra sem o gene exógeno. As amostras de solo foram coletadas mensalmente ao longo do ciclo da cultura. A quantidade de fungos no solo foi obtida por cultivo em placas de Petri, em triplicata, utilizando meio de cultura de Martin e diluições seriadas de solo. A partir dos isolados, as morfoespécies foram identificadas por amplificação e sequenciamento da região ITS (Internal Transcribed Spacer) do rDNA. O número de unidades formadoras de colônia de fungos e a riqueza de espécies não foram diretamente influenciados pelo tipo de algodão cultivado ou pelos diferentes estádios de desenvolvimento da cultura. Da mesma forma, o cultivo de algodão Bt não afetou a química do solo, embora tenham sido observadas diferenças em relação aos diferentes períodos de amostragem. De modo geral, nossa pesquisa contribui para o conhecimento atual sobre a agricultura em áreas tropicais, mostrando que não há evidências aparentes de efeito do algodão Bt sobre as comunidades fúngicas e a química do solo.
Palavras-chave: Algodão transgênico; Microbiota; Diversidade; Ecologia do solo; Fungos.

Resumen
El algodón transgénico se cultiva en Brasil desde 2005, incluyendo plantas que expresan el gen *cry*, derivado de la bacteria entomopatógena *Bacillus thuringiensis* (Bt). Esta tecnología se ha considerado eficiente para el control de insectos plaga objetivo. Sin embargo, se sabe poco sobre si estas plantas transgénicas pueden afectar los hongos del suelo y la química del suelo. Este estudio tuvo como objetivo evaluar los efectos del algodón genéticamente modificado resistente a insectos sobre la comunidad fúngica del suelo y la química del suelo. El experimento se realizó en el área agrícola de la Universidade Federal da Grande Dourados, municipio de Dourados, estado de Mato Grosso do Sul, Brasil. Se plantaron dos cultivares de algodón (*Gossypium hirsutum* L.; Malvales: Malvaceae), una que expresaba un gen de *Bt* y otra sin el gen exógeno. Las muestras de suelo se recolectaron mensualmente a lo largo del ciclo del cultivo. La cantidad de hongos en el suelo se determinó mediante cultivo en placas de Petri, por triplicado, utilizando el medio de cultivo de Martin y diluciones seriadas de suelo. A partir de los aislados, las morfoespecies se identificaron mediante la amplificación y secuenciación de la región ITS (Internal Transcribed Spacer) del rDNA. El número de unidades formadoras de colonias de hongos y la riqueza de especies no fueron directamente influenciados por el tipo de algodón cultivado ni por las diferentes etapas de crecimiento. Del mismo modo, el cultivo de algodón Bt no afectó la química del suelo, aunque se observaron diferencias entre los distintos tiempos de muestreo. En general, nuestra investigación contribuye al conocimiento actual sobre la agricultura en zonas tropicales, mostrando que no hay evidencia aparente del efecto del algodón Bt sobre las comunidades fúngicas y la química del suelo.
Palabras clave: Algodón transgénico; Microbiota; Diversidad; Ecología del suelo; Hongos.

1. Introduction

Genetically modified plants resistant to insects are an alternative for the control of agricultural pests. Bt cotton (Gossypium hirsutum L.) (Malvales: Malvaceae) is one of the most planted among insect-resistant transgenic crops grown in Brazil, together with maize (Zea mays L.) (Cyperales: Poaceae) and soybean [Glycine max (L.) Merr.] (Fabales: Fabaceae) (James, 2015; García et al., 2023; Razzaq et al., 2023; Freitas et al., 2024).

Transgenic Bt cotton possesses the gene cry, derived from the entomopathogenic bacterium Bacillus thuringiensis Berliner, 1911 (Bacillales: Bacillaceae), which encodes Cry 1Ac protein production. This crystal protein, when ingested by some caterpillars, is solubilized by alkaline pH (9.5) in their intestinal tract and cleaved by intestinal proteases, so that it is transformed into smaller peptides that bind to specific receptors on the epithelium and initiate a tissue lysis process, which ultimately leads to insect death. Insertion of this gene into the genome of the cotton plant confers resistance to some species of Lepidoptera, e.g., cotton leafworm - Alabama argillacea (Hübner, 1818) (Lepidoptera: Noctuidae), and pink bollworm - Pectinophora gossypiella (Saunders, 1844) (Lepidoptera: Gelechiidae) (Ortiz & Sansinenea, 2023).

Transgenic Bt plants initially seemed to provide economic and environmental benefits by reducing insecticide use, and in the first years after planting this technology has been efficient for controlling pests (Kathage & Qaim, 2012). However, recently research has reported problems of insect resistance after continued use of Bt crops (Gassmann & Reisig, 2023; Li et al., 2023; Tabashnik et al., 2023). Nevertheless, less attention has been paid to the effects of Bt plants on soil microbiota. The

potential effect of Bt crops on soil non-target organisms must be considered, as protein is expressed constitutively in all parts of the plant (Majumder et al., 2025) so that both the vegetable residues produced during plant development and remaining after harvest, as well as the exudates released by roots of plants during growth, may contain Bt toxin and could be incorporated into the soil (Saxena & Stotzky, 2003).

Microorganisms are essential for the functioning and balance of ecosystems. The processes of soil aggregation, organic waste decomposition, nutrient mineralization, pest and disease control, and establishment of symbiotic relationships are all performed with the effective participation of fungi (Gadd, 2007; Khatri et al., 2023). These microorganisms also participate in the degradation processes of pesticides (Fenner et al., 2013; Chakraborty et al., 2025) and can act in the compartmentalization of heavy metals in the soil, reducing their toxicity to the environment (Wu et al., 2010; Li et al., 2024).

Research has shown that Bt transgenic plants have a higher concentration of lignin in plant tissues, causing a slower decay rate (Flores et al., 2005; Lebedev et al., 2023), which could increase the exposure of soil microorganisms to the toxin and also favor the selection of resistance in target organisms (Saxena & Stotky, 2001).

This study aimed to evaluate the effects of genetically modified cotton resistant to insects on the soil fungal community and soil chemistry. It searched to determine the effect of Bt cotton on the soil fungal community, in comparison with a non-Bt cotton crop, by evaluating the: i) quantitative analysis in colony forming units (CFUs), and ii) analysis of fungal diversity through growing in culture medium and molecular identification of isolates. To the best of our knowledge, this is the first investigation on the effects of Bt cotton on soil fungal communities in Brazil.

2. Methodology

An experimental, field investigation was carried out in a quantitative study (Pereira et al., 2018) using descriptive statistics using data classes and absolute frequency values (Shitsuka et al., 2018) and statistical analysis (Vieira, 2021).

The cotton cultivars were grown in the agricultural area of the Experimental Farm of Universidade Federal da Grande Dourados (UFGD), Dourados, Mato Grosso do Sul, Brazil (Latitude 22°11′53″S, Longitude 54°55′59″W, and altitude of 430 m), during the 2009/2010 agricultural year.

The sample area comprised two fields with 5,000 m² each, where the treatments evaluated, Bt-cotton cultivar (NuOpal Bollgard) and its conventional non-Bt isoline (Delta Opal), were planted, with similar agronomic characteristics. A direct-planting system was used, where a millet (*Pennisetum glaucum* L.) (Cyperales: Poaceae) cultivar BRS 1501 was the preceding crop. Planting was performed at a density of 10 to 14 seeds per linear m and spacing between rows of 0.90 m. Both areas received the same fertilizing management: Base fertilization was applied at a rate of 450 kg.ha-1 of the formulation (N-P2O5-K2O, 08-20-20 + 0.3% Zn), and at 35 d after plant emergence 150 kg.ha-1 of urea (45% N) was applied. No phytosanitary products were applied during the period of soil sample collection.

Sampling for chemical and physical analysis of the soil, as well as analysis of the fungal community by growth in petri dishes were performed during the entire vegetative and reproductive plant development period, totaling six assessments: the first was 1 d before sowing (d 0), the second at 30 d after sowing (DAS), the third at 60 DAS, the fourth at 90 DAS, the fifth at 120 DAS, and the sixth at 150 DAS. The first sampling determined chemical and biological soil features before planting the cotton cultivars. A completely randomized design was used, consisting of three repetitions at distinct and random points. The soil used in the analysis was taken from the rhizosphere and rhizoplane.

The active microbiota in the form of colony forming units (CFU.ml-1) were evaluated by the plating technique, in triplicate, and also by inoculation of serial suspensions using specific growth media. Counting of fungal CFU's was performed using Martin's medium (Martin, 1950), consisting of the following substances: potassium phosphate (1.00 g), magnesium

sulfate (0.50 g), bacteriological peptone (5.00 g), glucose (10.00 g), agar (15.00 g), rose bengal (0.03 g), and distilled water qsp (1,000 mL). The rose bengal stain was dissolved in 10 mL distilled water before being added to the medium. A 1% streptomycin sulfate solution (30 mg/L of medium) in ethyl alcohol 96 GL was also added to the medium just before pouring into plates at 45–50°C. An amount of 10 g was used from each sample, which was carefully ground in a sterile mortar followed by the addition of 90 mL of a sterilized saline solution. The resulting slurry was poured into a sterile Erlenmeyer flask and stirred vigorously for 5 min. This suspension was diluted 10-4 times (adapted from Neder, 1992). After inoculation of 0.1 mL of each dilution per plate, these were inverted and incubated at 25°C for 7 d. The results obtained from counting of CFU's and discrimination of morphospecies were compared between the two treatments; and the fungi cultivated were isolated according to their morphocultural characteristics. The microculture method was also to determine the morphological characteristics.

To verify if the amount of fungi differed between cotton cultivars and collection months, data were subjected to a one-way analysis of variance by the F-test, and the means compared using analysis of variance (ANOVA), followed by a Tukey's honest significant difference test ($\alpha = 0.05$).

Molecular identification of the isolates was performed at the Laboratory of Biochemistry of Microorganisms and Plants (LBMP), Department of Technology, São Paulo State University (Universidade Estadual Paulista - UNESP), Jaboticabal Municipality, São Paulo State, Brazil. Determination of fungal species was based on comparing morphological characteristics of cultural growth such as color, relief, texture, and presence of pigment. A specimen of each morphospecies was chosen for molecular identification. The first step in DNA extraction was the cultivation of isolates in PD liquid medium (potato and dextrose), which remained in an incubator at 25°C for one week. After this period, fungi were sieved and washed to remove the culture medium residue, dried on paper towel, and macerated with liquid nitrogen until a powder formed. The macerated mycelia were placed in 2 mL tubes and supplemented with 750 µL previously heated extraction buffer (100 mM Tris pH 8.0, 50 mM EDTA pH 8.0, 500 mM NaCl, 1% SDS sodium dodecyl sulfate). Next, the tubes were placed in a water bath at 65°C for 40 min, being agitated every 10 min. After reaching room temperature of 25 ± 2°C, 400 µL potassium acetate was added to the tubes, which were carefully inverted and left on ice for 30 min. Subsequently, they were centrifuged at 12,000 rpm for 10 min at 10°C. Then, the resulting supernatants were transferred to new tubes. To this 700 µL chloroform [isoamyl alcohol solution (24:1)] was added, followed by careful stirring. This was followed by centrifugation again for 10 min. The supernatant was collected and supplemented with 400 µL chloroform:isoamyl alcohol solution (24:1), followed by repeating the same steps of stirring, centrifugation, and supernatant transfer. The tubes were left at -20°C overnight, and after this period they were centrifuged again at 12,000 rpm and 10°C for 15 min. The supernatant was discarded and 700 µL 70% ethanol was added to the pellet. The tubes were then centrifuged for 10 min and the supernatant discarded. The resulting pellet was dried, followed by the addition of 50 mL TE (Tris-EDTA) 10:1 (Kuramae-Izioka, 1997).

The amount of DNA and degree of purity were estimated by optical density in a spectrophotometer (NANODROP ND-1000) by absorbance. Each DNA extract was diluted to a concentration of 10 ng DNA/µL of suspension.

The extracted DNA was amplified using a polymerase chain reaction (PCR). For amplification of the ITS1-5 and 8S-ITS2 regions, the primers used were ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') / ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (White et al., 1990) to acquire a fragment of about 650 bp. The PCR reactions were performed using a 1X buffer (KCl 50 mM, Tris-HCl 200 mM, pH 8.4), 0.4 µL dNTPs, 1.5 U Taq DNA polymerase, 50 mM MgCl2, 5 pmol of each primer, 60 ng DNA, and sterile purified water (qsp 20 µL).

DNA was amplified in a thermocycler (PTC-100) with an initial cycle of 95°C for 4 min, 35 cycles at 94°C for 1 min, 60°C for 1 min, and 72°C for 90 s, with a final 10 min cycle at 72°C. The amplified DNA fragment of each isolate was then subjected to PCR sequencing using the DYEnamic ET Dye Terminator Kit (GE Healthcare), according to manufacturer's

instructions. The thermal cycling program was performed as follows: an initial cycle of 94°C for 2 min, 39 cycles at 94°C for 1 min, 60°C for 1 min for the reaction with primer ITS-1, and 52°C for ITS-4, and 72°C for 90 s, with a final cycle of 10 min at 72°C.

The electropherograms obtained were visualized and analyzed using the ABI Analysis Data Collection software and converted to nucleotide sequences using the DNA Sequencing Analysis Software Version 3.3. These electropherograms generated by the sequencing process were subjected to the software package Phred/Phrap/Consed (Gordon et al., 1998), to check its quality, alignment, cut of the extremities, and removal of the vector. As DNA of the isolates was sequenced in duplicate, a local alignment was performed to confirm the sequence. The sequences obtained were compared with the database using the BLAST tool (Altschul et al., 1997) to verify their similarity in relation to sequences already deposited in the database of the National Center for Biotechnology Information (NCBI, Genbank). Phylogenetic analyses were performed using the software MEGA5 (Tamura et al. 2011), based on the neighbor-joining method (Saitou & Nei, 1987); the tree was constructed with 1,000 replicates for obtaining the bootstrap values (Felsenstein, 1985). Evolutionary distances were calculated using the method of Jukes & Cantor (1969). All sequences were deposited in Genbank.

The Shannon diversity index was used to evaluate the diversity of fungal colonies in soil with the cultivation of Bt or non-Bt cotton, as described by Magurran (2013): $H' = - \Sigma pi. \ln pi$, where pi is the proportion of individuals encountered belonging to species i, utilizing the software DiVes (Rodrigues, 2005).

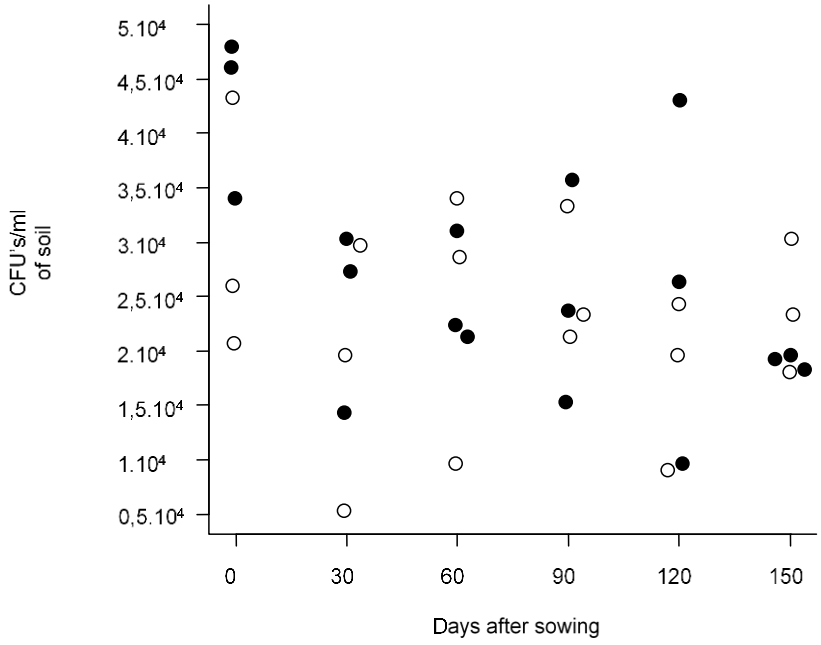
Chemical characteristics of the soil samples were measured at the Laboratory of Soils, Faculty of Agricultural Sciences, UFGD. The following chemical analyses were performed: pH determination, analysis of H+Al (exchangeable acidity), Ca, Mg, P, K, sum of bases (SB), cation exchange capacity (CEC) at pH 7.0, percentage of base saturation (V), and soil organic matter (SOM), according to the procedure proposed by Silva (2009). Micronutrient analyses (Cu, Fe, Mn, and Zn) were performed via Mehlich-1 extraction. Particle-size distribution of soils was determined in the laboratory using the pipette method, with dispersion of 20 g ADS (air dried soil) with sodium hydroxide 1 mol L-1. Sand was separated in sieves with mesh measuring 0.053 mm in diameter and fractionated. Clay was separated by sedimentation according to Stokes' law, and silt was determined as the total minus the clay and sand fractions.

To obtain a gradient in few dimensions composed of the soil features, a principal component analysis (PCA) was used to sort the samples collected monthly for the Bt and non-Bt cotton cultivars. To investigate if the time elapsed since planting and cotton cultivar explained this ordinance, a multivariate analysis of variance (MANOVA) was performed considering the Pillai Trace statistic. To determine whether chemical and textural characteristics differed among cotton cultivars and between collection months, data were subjected to one-way analysis of variance using the F-test, and the means were compared by Tukey's honest significant difference test ($\alpha = 0.05$).

3. Results and Discussion

The average number of colony forming units (CFU's) obtained from fungi of soil cultivated with Bt and non-Bt cotton did not differ significantly ($F = 1.39$; $df = 1$; $P = 0.25$). Significant differences were also not found regarding the different sampling periods ($F = 2.21$; $df = 5$; $P = 0.08$) (Figure 1). Therefore, it is possible to infer that the amount of fungi in the soil was not directly influenced by the cotton cultivar or sampling period.

Figure 1 - Average number of colony forming units in soil, with cultivation of Bt (open circles) and non- Bt cotton (filled circles), during 150 days after sowing.



Source: Authors.

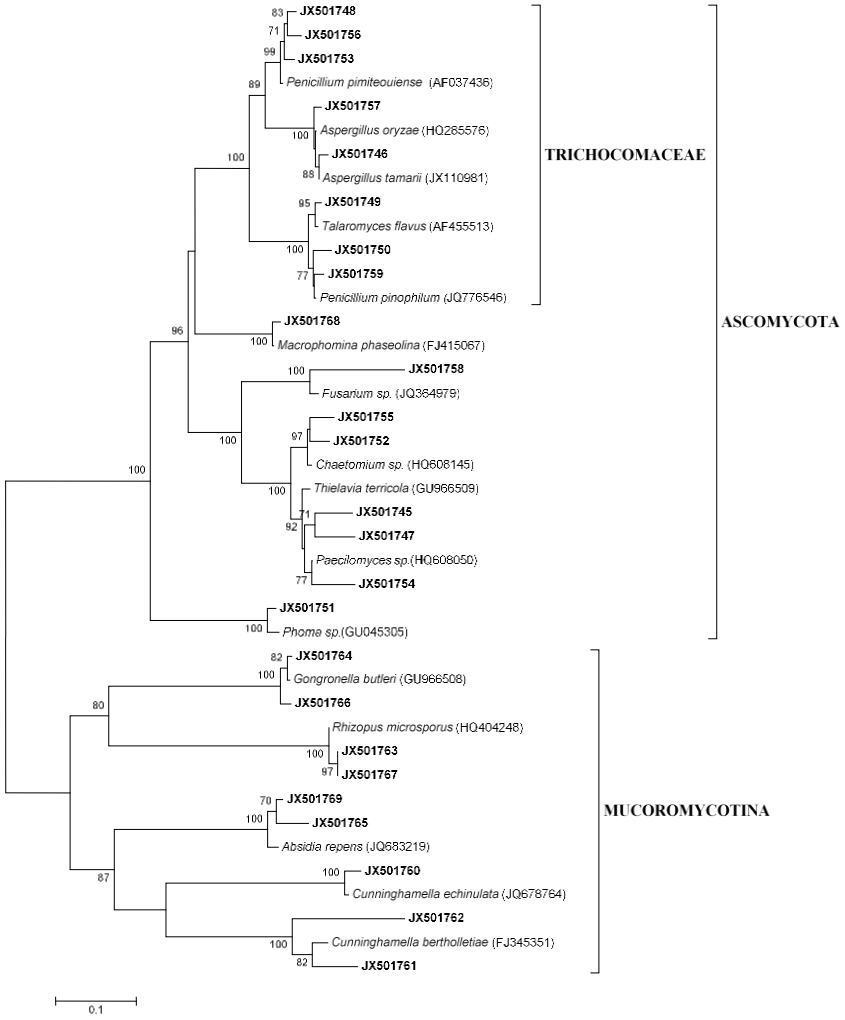
The results obtained in this study confirm those found by other authors, in which Bt transgenic crops displayed no significant effects on different groups of soil microorganisms, including fungi (Flores et al., 2005; Shen et al., 2025). However, some studies indicated the interference of Bt cotton toxins on soil microbiota. Chen et al. (2016) investigated influence of Bt cotton on the early stages of arbuscular mycorrhizal (AM) fungal life cycle, Rhizophagus irregularis, and concluded that the Bt trait significantly contributed to the inhibition of pre-symbiotic development and AM fungal colonization, suggesting it might be attributed to either Bt toxin toxicity or interference of signal perception between AM fungi and the hosts. Similarly, Fazal et al. (2024) reported that dual Bt-transgenic maize had a significant impact on soil microbial communities, including fungi. In contrast, Ge et al. (2025) found through metagenomic analyses that the composition of rhizosphere bacterial communities varied significantly among Bt rice cultivars, whereas fungal and nematode communities remained unaffected.

A total of 1,935 colonies were observed in all samples and dilutions, where it was possible to differentiate 45 morphospecies by analyzing the morphological and cultural characteristics. Thirty morphospecies were common to the soil of both Bt and non-Bt cotton, three morphospecies were found only in soil planted with non-Bt cotton, and twelve morphospecies observed only in the soil with Bt cotton. The sequenced and identified isolates are shown in Figure 2 and are considered common inhabitants of soil in areas planted with cotton crops (Carvalho, 2008; Cui et al., 2024).

The phylogenetic tree constructed with the sequences identified (Figure 2) revealed the presence of six species from the Trichocomaceae family, two species from the Chaetomiaceae family, three species from the Mucoraceae family, two species from the Cunninghamellaceae family, one species from the Nectriaceae family, one species from the Botryosphaeriaceae family, and one species from the Pleosporaceae family. The species identified belong to the phylum Ascomycota and subphylum Mucoromycotina. The latter belonging to the phylum Zygomycota; however, after advances in phylogenetic analyses, it was found that it was a polyphyletic group, where the subphylum Mucoromycotina was considered one group with uncertain taxonomic position (Stajich et al., 2009). The phylum Ascomycota is the largest taxon in the fungal

kingdom, comprising approximately 64% of all species described which occupy the most diverse niches including saprobes, mutualists, and parasites (Stajich et al., 2009; Mondo & Grigoriev, 2025).

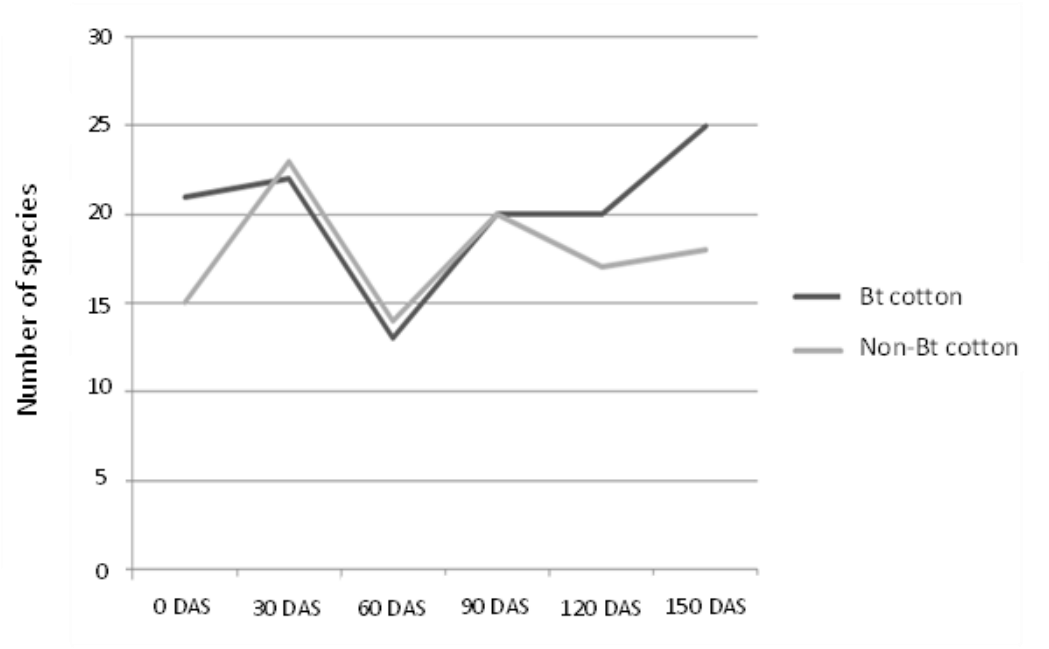
Figure 2 - Phylogenetic tree constructed with the sequences obtained from the isolates (in bold) and species with greater similarity in the Genbank database, through the model of neighbor-joining reconstruction.



Source: Authors.

The fluctuation of species number in both treatments is shown in Figure 3. Similar behavior was observed for the fungal community in soil cultivated with Bt and non-Bt cotton. The greatest difference in the number of morphospecies occurred in the last sampling (150 DAS), where the fungal community showed greater richness in soil with planting of Bt cotton, with the presence of 25 morphospecies. Soil with non-Bt cotton had highest amount of morphospecies at 30 DAS, with 23 morphospecies (Figure 3). It is noteworthy that the fluctuation in species number was virtually identical between Bt and non-Bt cotton, and the few differences observed are possibly owing to seasonal variation. Corroborating this, Shannon's diversity index revealed similarity between soil fungi obtained from the area cultivated with Bt cotton (H' = 3.98) when compared to the soil of non-Bt cotton (H' = 3.92).

Figure 3 - Fluctuation in the number of species of the fungal community in soil planted with Bt cotton and non Bt cotton along the sampling period, in days after sowing (DAS).



Source: Authors.

Some studies have shown that fungal biomass, diversity, and community structure were not significantly altered or affected by transgenic plants (Lamarche et al., 2011; Berini et al., 2024; Xie et al., 2025). Conversely, Vadakattu & Watson (2004) performed a detailed scanning electron microscopy analysis, which showed an intense fungal colonization in Bt cotton residues, whereas in the soil planted with non-Bt cotton a lower number of fungi was reported. The authors also observed that the fungal community was quite different with respect to species composition.

All variables displayed significant differences (Table 1), with the exception of the amount of soil organic material (SOM), zinc content, base saturation index (V%), and percentage of organic carbon (C%), for which significant differences were not found among sampling times (DAS) (data not shown).

Overall, Bt cotton caused no effect on the chemical variables assessed, once there was no difference between Bt and non-Bt cotton within any sampling time. Thus, differences were reported only considering the different sampling times (Table 1).

Table 1 - Chemical characteristics of the soil samples analyzed in the two treatments, Bt and non-Bt cotton, during cotton development in days after sowing (DAS).

	0 DAS		30 DAS		60 DAS		90 DAS		120 DAS		150 DAS	
	Bt	n Bt	Bt	n Bt	Bt	n Bt	Bt	n Bt	Bt	n Bt	Bt	n Bt
pH H_2O	6.26^{a}	5.93^{a}	5.89^{b}	5.42^{b}	6.06^{ab}	5.87^{ab}	6.35^{a}	5.96^{a}	6.15^{a}	5.93^{a}	6.08^{a}	5.98^{a}
P (mg/dm^3)	41.05^{a}	46.61^{a}	23.38^{ab}	16.68^{ab}	11.60^{b}	10.46^{b}	33.02^{ab}	36.37^{ab}	17.62^{ab}	17.49^{ab}	20.37^{ab}	24.58^{ab}
K (mmol)	12.07^{ab}	8.87^{ab}	7.45^{b}	7.97^{b}	3.52^{b}	3.60^{b}	21.35^{a}	17.17^{a}	6.48^{b}	6.19^{b}	7.62^{b}	10.68^{b}
Al (mmol)	1.23^{a}	6.54^{a}	2.04^{b}	1.63^{b}	1.23^{b}	1.23^{b}	1.23^{b}	1.23^{b}	1.23^{b}	2.86^{b}	1.63^{b}	2.86^{b}
Ca (cmol)	6.17^{a}	6.50^{a}	5.73^{ab}	5.50^{ab}	6.43^{ab}	5.90^{ab}	5.67^{ab}	6.73^{ab}	5.30^{b}	4.77^{b}	5.17^{ab}	5.77^{ab}
Mg (cmol)	2.80^{ab}	2.70^{ab}	2.40^{abc}	2.57^{abc}	2.60^{bc}	2.30^{bc}	2.70^{a}	3.00^{a}	2.37^{c}	2.17^{c}	2.30^{abc}	2.73^{abc}
H+Al (cmol)	4.54^{a}	5.85^{a}	3.97^{ab}	5.10^{ab}	3.97^{ab}	4.28^{ab}	4.28^{ab}	5.04^{ab}	3.91^{b}	4.14^{b}	4.59^{ab}	5.38^{ab}
SB	101.74^{ab}	100.87^{ab}	88.78^{bc}	88.64^{bc}	93.85^{bc}	85.60^{bc}	105.02^{a}	114.50^{a}	83.14^{c}	75.52^{c}	82.28^{bc}	95.68^{bc}
CEC	147.10^{ab}	159.37^{ab}	128.47^{abc}	139.64^{abc}	133.53^{bc}	128.44^{bc}	147.85^{a}	164.91^{a}	122.28^{c}	116.93^{c}	128.22^{abc}	149.48^{abc}
Cu (mg/dm^3)	17.85^{a}	19.97^{a}	20.32^{a}	15.78^{a}	20.23^{ab}	11.63^{ab}	17.25^{a}	17.10^{a}	13.04^{bc}	9.54^{bc}	9.29^{c}	6.55^{c}
Mn (mg/dm^3)	114.62^{a}	122.77^{a}	96.90^{ab}	106.45^{ab}	100.73^{bc}	61.97^{bc}	124.65^{a}	132.30^{a}	92.79^{bc}	73.02^{bc}	71.58^{c}	55.22^{c}
Fe (mg/dm^3)	60.49^{ab}	45.07^{ab}	84.34^{a}	46.81^{a}	77.93^{a}	47.05^{a}	58.67^{ab}	42.46^{ab}	64.15^{ab}	38.40^{ab}	51.37^{b}	22.50^{b}
Sand(%)	10.76^{c}	11.93^{c}	10.66^{ab}	12.21^{ab}	13.98^{c}	11.34^{c}	11.12^{a}	11.75^{a}	11.05^{bc}	11.63^{bc}	11.41^{a}	12.47^{a}
Clay(%)	68.36^{cd}	66.23^{cd}	64.53^{d}	64.05^{d}	57.06^{cd}	70.41^{cd}	72.20^{bc}	70.86^{bc}	74.86^{ab}	78.81^{ab}	76.91^{a}	78.53^{a}
Silt(%)	20.88^{ab}	21.84^{ab}	24.81^{ab}	23.74^{ab}	28.96^{a}	18.25^{a}	16.68^{abc}	17.39^{abc}	14.10^{bc}	9.56^{bc}	11.67^{c}	9.0^{c}

SB – sum of exchangeable bases (Ca, Mg, K, H+Al); CEC – cation exchange capacity; Equal letters on the same line do not statistically differ between sampling periods, independent of the treatments, by the Tukey's test ($p \leq 0.05$). Source: Authors.

Evaluation of the soil chemical properties is very important to understand fungal community structure. For instance, changes in the soil pH under natural conditions alter the distribution of fungal species (Cabello & Arambarri, 2002; Xiong et al., 2024).

4. Conclusion

Bt cotton apparently shows innocuity on soil fungi community and chemical properties. The next steps of this research should evaluate the effects of different Bt cotton toxins in different Brazilian states, once there is a variety of Bt proteins, as Brazil displays different climate conditions which may significantly influence the results.

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