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ARTICLE

Diversity of filamentous fungi in Cerrado soil under native vegetation

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ABSTRACT: (Diversity of filamentous fungi in Cerrado soil under native vegetation). Soil is a habitat intrinsic to the development of the biodiversity of microorganisms, including fungi. The municipality of Tangará da Serra, MT, is located in the Brazilian cerrado region, as well, considering the limited knowledge of the fungal biodiversity in this region, this study aimed to isolate and identify filamentous soil fungi through seasonal surveys in three areas of native vegetation in the Cerrado. Three samples were collected during the wet period and three in the dry period. The samples were collected at a depth of 20 cm, each point composed 25 grams of soil, and each sample was subjected to serial dilution technique, inoculated in dishes, placed in a bacteriological incubator at 28 ºC for five days. Were isolated from 136 specimens (25 *Mycelia sterilia*) dispersed in 17 genera, equivalent to 02 Ascomycota, 04 Zygomycota and 11 Deuteromycota these subdivided into 10 genera of Hyphomycetes and 01 genera of Coelomycetes. The highest abundance occurred in the wet season (69 specimens) compared to drought (67 specimens) and a greater richness was observed in the dry season (12 genera) compared to the wet season (11 genera).
Key words: identification, diversity, Deuteromycota.

RESUMO: (Diversidade de fungos filamentosos no solo do Cerrado sob vegetação nativa). O solo é um habitat intrínseco para o desenvolvimento da biodiversidade de microrganismos, destacando-se os fungos. O município de Tangará da Serra, MT, está localizado na região de cerrado brasileiro, desta forma, considerando o escasso conhecimento da biodiversidade fúngica nesta região, o presente trabalho objetivou isolar e identificar fungos filamentosos do solo, através de levantamentos sazonais em três áreas de vegetação nativa de cerrado. Foram coletadas três amostras no período chuvoso e três no período de estiagem. As sub-amostras foram coletadas a uma profundidade de 0 a 20 cm do solo, cada amostra correspondia a 25 gramas de solo, posteriormente submetidas à técnica de diluição seriada, inoculada em placas e incubadas em estufa bacteriológica a 28 ºC durante cinco dias. Foram isolados 136 espécimes (25 *Mycelia sterilia*) disseminados em 17 gêneros, sendo 02 Ascomycota, 04 Zygomycota e 11 Deuteromycota estes subdivididos em 10 gêneros de Hyphomycetes e 01 gênero de Coelomycetes. A maior abundância ocorreu no período chuvoso (69 UFC) em relação a estiagem (67 UFC) e a maior riqueza foi observada na estiagem (12 gêneros) em analogia ao período chuvoso (11 gêneros).
Palavras-chave: Identificação, Diversidade, Deuteromycota.

INTRODUCTION

The soil is the junction of minerals, organic matter, air and water that allows biological activity in the environment. Compared to other terrestrial habitats, "the soil in their nature complex and heterogeneous dynamics, allows organisms with different metabolisms live side by side, interacting in a state of dynamic equilibrium, providing ideal conditions for an extremely high biodiversity" (Moreira & Siqueira 2006).

Soil is an excellent place to develop diverse populations of microorganisms, including fungi, establishing a reservoir of microbial genetic diversity. With its large amount of organic debris, is an inexhaustible source of food for these organisms, which can be found from the surface to a depth of 15 to 20 centimeters (Tsai *et al.* 1992, Silveira 1996).

Fungi are eukaryotic organisms and, factors such as humidity, temperature, pH and oxygen influence its growth. Their life cycle starts from propagules generated in sexuallly and asexually order or fragments of hyphae (Alexopoulos 1996, Bernardi & Nascimento 2005). Due to the varying morphology, such as the arrangement of spores, the characteristics of sporophores, the presence of ornaments and other special modifications of the hyphae, it is possible to perform identification through taxonomy (Bernardi & Nascimento 2005).

Studies on soil fungi extend to the vast biomes found in Brazil, but in the Cerrado the biodiversity of these organisms has not been a subject of discussion, resulting in a lack of knowledge on the subject (Silva 2006).

Occupying an area of approximately two million square kilometers, the Cerrado is home to a large number of microscopic communities, reaching the expectation 60–100 thousand species of fungi, representing 5–7% of the mycoflora of the globe (Dianese *et al*. 2001, Silva *et al*. 2010).

The Cerrado has a tropical climate characterized by two distinct seasons, the dry season, which extends from April to September, with temperatures averaging around 27 °C, and the wet season from October to March, with

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an average temperature of 22 °C and an annual rainfall average of 1500 mm (Silva 2006).

According to Alexopoulos (1996), the optimum temperature for most fungal species, termed mesophilic fungi, is between 25 and 30 °C. Lacaz *et al.* (2002) assert that mesophilic fungi are medically important, and the saprophytic or mycelial phase is the infective form found in the soil.

Fungi from an ecological aspect are important in the food chain, performing nutrient cycling, supporting plant life from mutualistic symbiotic associations with the roots, and supporting the increased absorption of nutrients through this extension of the plant root system. Through breaking down the cellulose, fungi decompose the plant, absorb carbon and return phosphorus and other minerals (Van Der Heijden *et al.* 1998, Ruegger & Tauk-Tornisielo 2004).

Therefore, the objective of this work was to isolate and identify fungi species through a survey in native Cerrado vegetation in the city of Tangará da Serra - MT, considering the limited knowledge of mycobiota in this biome.

MATERIAL AND METHODS

Study area and sampling

The city of Tangará da Serra, in the State of Mato Grosso, has a tropical ,wet hot and humid climate, with two well-defined periods: the wet season during September to April and the dry season between May and August (annual rainfall varies from 1,300 to 2,000 mm). The temperature ranges from 16 to 36 ºC with relative humidity of 80% (Prefeitura Municipal de Tangará da Serra 2007). The soil sampling was made seasonally in three areas (A, B, C) of similar native vegetation, respectively: São Marcelo Farm (14°38'27.33 "S, 57º51'38.04"W), Bahia Farm (14°37'27.15"S, 57º25'15.84"W) and Paraíso Farm (14º40'7.91"S, 57°23'14.15"W).

In each area of native vegetation, we collected two samples, one in the dry season and another in the wet season, totaling six samples in the three areas (A, B, C). With the aid of a GPS device, we recorded all places of sampling, so we could perform the two samples approximately in the same location.

The soil samples consisted of five subsamples with approximately 100 to 150 g, each. With the aid of a shovel, we first removed the litter layer, and then collected the soil samples at a depth of 0–20 cm. Subsequently, the soil samples were packed in sterilized polyethylene bags, and transported in ice coolers to the Laboratory of Microbiology of the University of the State of Mato Grosso (UNEMAT). We collected 5 grams of each subsample, comprising a sample of 25 g per area (Machado 2003).

Isolation, identification and storage

For each sample consisting of 25 g, the serial dilution technique was performed in accordance with Tortora *et al*. (2005). Later in this process, we inoculated 1.0 ml of suspensions 10^{-2}, 10^{-3} and 10^{-4}, in Petri dishes containing PDA (potato-dextrose-agar) culture medium and 500 $mg.L^{-1}$ of chloramphenicol for inhibition of bacterial contamination and incubated the plates in a bacteriological incubator (BOD) at 28 °C for five days.

For the identification of the fungi, we transferred a small portion to Petri dishes containing PDA and incubated this in BOD at 28 ºC for a period of three days. After the incubation period we identified the specimens from the macro and microscopic features of the colonies according to Larone (1995), Barnett & Hunter (1998) and Lacaz *et al*. (2002) and, when necessary, we performed the microculture slide (Lacaz-Ruiz 2000). For fungi that did not show reproductive structures, we used alternative means (oats and cornmeal agar) and incubated for four days for further identification.

For the storage of each specimen identified, we transferred a small portion to two test tubes containing PDA culture medium and 4% oatmeal agar and incubated this in BOD at 28 ºC. After satisfactory growth, we sealed and stocked the sample at a temperature of 4 ºC in order to produce a mycology collection of fungi of the Cerrado at the UNEMAT.

Data analysis

We analyzed the data using Margalef, Simpson, Shannon–Wiener species richness indices, and the abundance of colony-forming units using the DIVES statistical program (Rodrigues 2005).

RESULTS AND DISCUSSION

Of the samples analyzed in the three areas of Cerrado during the wet season and drought, we obtain 136 colony-forming units (CFU), distributed in 17 genera, allocated in subdivisions Ascomycota (02 CFU), Zygomycota (04 CFU) and Deuteromycota (11 CFU), this latter subdivided into classes: Hyphomycetes (10CFU) and Coelomycetes (01CFU) (Table 1).

Of the total of isolated specimens, we identified 18% (25 CFU) as *Mycelia sterilia* (Agonomycetales) according to Hawksworth (1983), who states that this is a large group of fungi of various morphological types, which share the characteristic of not having production spores.

The genera with a higher dominance in the total fungi isolated, according to the number of species were: *Penicillium* (28%), *Trichoderma* (18%), *Mucor* (11%) *Paecilomyces* (6%), *Aspergillus* (5%), *Cladosporium* (3%), *Acremonium*, *Fusarium*, *Rhizopus*, *Verticillium*, *Conidiobolus*, *Cunninghamella*, *Gliocladium*, *Humicola*, *Periconia*, *Phoma* and *Pyrenochaeta* (1%).

Among the isolated genera, six are common in both periods (*Penicillium* sp., *Trichoderma* sp., *Mucor* sp., *Paecilomyces* sp., *Aspergillus* sp. and *Fusarium* sp.) From these data, we found that these fungi are ubiquitous, can adapt to temperature changes, but their propagation abundance is dependent on the ideal conditions of temperature and humidity.

The first three genera *Penicillium*, *Trichoderma* and *Mucor* were more abundant regardless of the season; however, they were considerably more abundant during

the wet season, which may indicate affinity with this period, while the fourth and fifth (*Paecilomyces* sp. and *Aspergillus* sp.) were more abundant during the dry season, indicating a preference that is contrary to the first three. For the last genre, *Fusarium*, there was no differentiation between the hydrological periods.

The incidence of *Penicillium* sp. in the wet season, with an average temperature of 22 ºC and rainfall between 1,300 to 2,000 mm, was higher (33%) than in the dry season (22%) which had an average temperature of 27 ºC. According to Lacaz *et al*. (2002) the optimum temperature for greater propagation of the genus *Penicillium* is 20–25 ºC, and this be found in many places, such as decaying organic matter, air and soil.

According to Silveira (1996), fungi have a wide range of behavior with respect to temperature as well as a resistance to cold and heat. This explains the common occurrence of fungi between the two climatic periods. The temperature influences not only the growth but also the vegetative part, quantity and size of the spores, which collaborates with the result of abundance of *Penicillium* sp. during the wet and dry seasons.

Just as some genera have a similar occurrence in both periods, other genres occurred only in specific periods, such as *Cladosporium*, *Acremonium*, *Rhizopus*, *Phoma*, *Pyrenochaeta* and *Conidiobolus*, which appeared only in the dry season. The genres such as *Humicola*, *Periconia*, *Cunninghamella*, *Gliocladium* and *Verticillium* were exclusive to the wet season, thus this also corroborates the findings of Silveira (1996) regarding the influence of temperature on the occurrence of species in different climatic periods.

Using the Shannon-Wiener index, we found that the diversity of species isolated in areas of native vegetation in the Cerrado (Table 02) indicate that the dry season has the highest diversity (H '= 0.91) when compared to the wet season (H '= 0.76) and can therefore conclude that the diversity of filamentous fungi of the rainy season is unlike that of the dry season.

In the Margalef index, which assesses the diversity of communities based on the numerical distribution of individuals (CFU) of different genres, depending on the total number of individuals (CFU), our results gave an index of 9.37 in the period of drought, and 9.00 in the wet season.

Prade *et al*. (2006), in studies on the diversity of soil filamentous fungi in a planting of *Hovenia dulcis* (Thunb), examined the species richness during the four seasons and presented a result showing a higher Margalef diversity index in winter, therefore having a correlation with the present study, since the region's winter (June to September) coincides with the dry season (April–September) of the Cerrado.

In the Simpson index (D') the period with the highest diversity was drought, with an index of 0.94 for diversity.

Among the three areas that we sampled during this period, the area A (Farm São Marcelo) was where we found the greatest diversity of fungi when we consider

Table 1. Frequency of colony-forming units (CFU) of filamentous fungi isolated from Cerrado soils under native vegetation (A, B, C) in the municipality of Tangará da Serra, MT, during the wet season (C) and dry season (E).

Genus	Collection point						CFU
	A. Farm São Marcelo		B. Farm Paraíso		C. Farm Bahia		
	C	E	C	E	C	E	
DEUTEROMYCOTA							
Hyphomycetes							
Aspergillus sp..	1	2	2	-	-	2	7
Acremonium sp.	-	1	-	-	-	1	2
Cladosporium sp.	-	3	-	-	-	1	4
Fusarium sp.	1	1	-	-	-	-	2
Gliocladium sp.	-	-	1	-	-	-	1
Humicola sp.	1	-	-	-	-	-	1
Paecilomyces sp.	1	1	1	3	1	1	8
Penicillium sp.	1	4	11	10	11	1	38
Trichoderma sp.	4	1	6	5	4	4	24
Verticillium sp.	-	-	2	-	-	-	2
Coelomycetes							
Phoma sp.	-	1	-	-	-	-	1
ZYGOMYCOTA							
Conidiobolus sp.	-	-	-	-	-	1	1
Cunninghamella sp.	1	-	-	-	-	-	1
Mucor sp.	6	1	2	1	2	3	15
Rhizopus sp.	-	1	-	1	-	-	2
ASCOMYCOTA							
Periconia sp.	1	-	-	-	-	-	1
Pyrenochaeta sp.	-	-	-	1	-	-	1
Mycelia sterilia	4	3	3	7	2	6	25
Total de UFC	21	19	28	28	20	20	136

Table 2. Genus richness (S), PlentyNumber of CFU (N), Margalef Index (Dα), Simpson (D ') and Shannon–Wiener (H') of filamentous fungi isolated from Cerrado soils under native vegetation in the municipality of Tangará da Serra, MT, in two different seasons.

Index	Collection point					
	1. Farm São Marcelo		2. Farm Paraíso		3. Farm Bahia	
	C	E	C	E	C	E
S	9	10	7	6	4	8
N	17	16	25	21	18	14
Dα	6,50	7,47	4,29	3,78	2,39	6,11
D'	0,85	0,92	0,76	0,72	0,62	0,89
H'	0,81	0,93	0,68	0,61	0,47	0,83

the Margalef index, with 6.50 in the wet season and 7.47 in the dry season. This result was obtained because this area is located closer to a body of water, since the humidity followed by temperature are key factors for the occurrence of the abundant spread of fungi.

The highest total abundance of the three areas occurred during the wet season, with 69 CFU, and the reatest richness was obtained in drought, with 12 genera. These results can be explained by the fact that rainfall own genus *Penicillium* as dominant, and richness in the dry season because it has a humidity and temperature conducive to the development of the species found.

The dominance of the fungi Deuteromycota (64%) obtained in this study occurs in several Brazilian ecosystems. These results corroborate with studies by Atili (1994) in Juréia-Itatins, Atlantic Forest region, with the occurrence of 82% individuals of this group; Antunes *et al*.'s (1993) studies in the Biological Reserve of Alto da Serra, Paranapiacaba, SP, who observed 38% predominance of these group, and Cavalcante *et al.* (2006) in isolations performed in the region of Xingó (northeast) during the dry and wet season, identifying 84% dominance of Deuteromycotas.

Studies aimed at identifying the Cerrado soil mycoflora are scarce. Seeking similarities with studies in other regions, we find the Xingó region, located in the Brazilian northeast, where Cavalcanti *et al*. (2006) showed results similar to those of our study, identifying genera such as *Penicillium*, *Aspergillus*, *Fusarium*, *Rhizopus*, *Trichoderma*, *Cladosporium*, *Humicola*, *Paecilomyces*, *Acremonium*, *Cunninghamella* and *Phoma*.

In enzyme activity studies of soil microorganisms in an ecological station in southwest Juréia-Itatins, SP, filamentous fungal species such as *Aspergillus*, *Penicillium* sp., *Mucor* sp., *Cladosporium* sp., *Gliocladium* sp., *Paecilomyces* sp., *Trichoderma* sp. and *Verticillium* sp. were isolated, which is also similar to the results obtained in this study (Ruegger & Tauk-Tornisielo 2004).

CONCLUSIONS

The soil of the Cerrado has a wide range of filamentous fungi. In the region's two well-defined seasons, we could observe a 51% occurrence of fungi in the dry season and 49% during the wet season. The soil fungi of the three identified areas of native Cerrado vegetation are mostly Hyphomycetes belonging to the genera *Penicillium* and *Trichoderma,* this genera having a high incidence in different Brazilian biomes.

The wet season has a considerably higher abundance of individuals than the dry season, a result that is contrary to the richness of genres found.

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