

Occurrence of filamentous fungi in yerba mate discarded in the environment

Ocurrencia de hongos filamentosos en yerba mate desechada en el medio ambiente

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ABSTRACT

Yerba mate, *Ilex paraguariensis* A. St. Hil. (Aquifoliaceae), waste is one of the most common byproducts generated in households in southern Brazil due to the traditional consumption of mate. However, the decomposition of this waste by fungi has not been thoroughly evaluated. In order to study the fungal succession in mate residue, 450 samples were evaluated during the autumn, winter, and spring, and kept for two months in a humid chamber (aerobic medium) for the growth of associated filamentous fungi. We morphologically identified the fungi by means of a microscopy device and taking into consideration only the surface-developed mycelia. Twenty-two genera of filamentous fungi were found, of which the Zygomycota (*Rhizopus* spp. and *Mucor* spp.) microflora deserve special attention since they were the only fungi growing in the first 19 days. After this period, the fungi Deuteromycota (mainly *Aspergillus* spp.) and Oomycota (*Pythium* spp.) develop more vigorously, supplanting the previous ones. The species of *Curvularia*, *Fusarium*, *Verticillium*, and *Pythium* are phytopathogens and end up having their inoculum source increased with the irregular disposal of this residue in nature. The presence of numerous fungal genera, which are known to be pathogenic according to the literature, highlights the importance of implementing proper waste disposal practices in the environment. Improper disposal of the yerba mate waste can result in contamination not only of yerba mate itself but also of other commercial crops. Therefore, it is imperative that greater attention be paid to the disposal of this waste.

Key words: *Ilex paraguariensis*; mycodiversity; phytopathology; residue; succession fungal; taxonomy.

RESUMEN

La yerba mate, *Ilex paraguariensis* A. St. Hil. (Aquifoliaceae), es uno de los subproductos más comunes generados en los hogares del sur de Brasil debido al consumo tradicional de mate. Sin embargo, la descomposición de este residuo por hongos no ha sido evaluada a fondo. Con el fin de estudiar la sucesión fúngica en el residuo de la yerba mate, se evaluaron 450 muestras durante el otoño, invierno y primavera, y se mantuvieron durante dos meses en una cámara húmeda (medio aeróbico) para el crecimiento de hongos filamentosos asociados. Se identificaron morfológicamente los hongos mediante un dispositivo de microscopía y teniendo en cuenta solo el micelio desarrollado en la superficie. Se encontraron veintidós géneros de hongos filamentosos, de los cuales la microflora de Zygomycota (*Rhizopus* spp. y *Mucor* spp.) merece especial atención, ya que fueron los únicos hongos que crecieron en los primeros 19 días. Después de este período, los hongos Deuteromycota (principalmente *Aspergillus* spp.) y Oomycota (*Pythium* spp.) se desarrollaron con más vigor, suplantando a los anteriores. Las especies de *Curvularia*, *Fusarium*, *Verticillium* y *Pythium* son fitopatógenas y terminan aumentando su fuente de inoculación con la disposición irregular de este residuo en la naturaleza. La presencia de numerosos géneros fúngicos, que se sabe que son patógenos según la literatura, destaca la importancia de implementar prácticas adecuadas de eliminación de residuos en el medio ambiente. La eliminación inadecuada de los residuos de yerba mate puede resultar en la contaminación no solo de la yerba mate en sí, sino también de otros cultivos comerciales. Por lo tanto, es imperativo prestar mayor atención a la eliminación de estos residuos.

Palabras clave: fitopatología; *Ilex paraguariensis*; micodiversidad; residuo; sucesión fúngica; taxonomía.

INTRODUCTION

Mate is a popular non-alcoholic hot beverage consumed in South America. *Ilex paraguariensis*, also called yerba mate, is the main feedstock for this drink. Its consumption is cultural, linked to history and tradition, dating back mid-1500s by native people called *Guaranis* (Zanin & Meyer, 2018). Its health benefits, such as stimulating effect, anti-inflammatory and antioxidant activity, are well known (Gullón *et al.*, 2018). Brazil is a significant producer and consumer of yerba mate, with 390 tons produced in 2017 (IBGE, 2018). In the state of Rio Grande do Sul, the per capita consumption is 9kg/year (IBRAMATE, 2018). Due to its high consumption, large amounts of waste are generated and its improper disposal may cause environmental issues and harm wildlife (Neves *et al.*, 2022).

Despite being a natural organic compound, the waste generated by the consumption of *mate* does not have an adequate destination yet. Research has been made exploring its potential applications as biodegradable films (Arrieta *et al.*, 2018), as a source of antioxidant phenolic compounds (Gullón *et al.*, 2018), and also in the reinforcement of epoxy-based composite materials (Neves *et al.*, 2022). However, its large-scale reuse is not yet a common reality, and most of the waste generated goes to landfills or home gardens (Gullón *et al.*, 2018). This is of concern because pathogens may be present in this waste, potentially causing harm to the environment.

Several studies have been done in the last decades exploring the relationship between fungi and yerba mate. Some works describe the pathogenicity of *Fusarium* to yerba

mate as the main causal agent of root rotting disease (Mezzomo *et al.*, 2021; Mezzomo *et al.*, 2019), and besides *Fusarium*, some authors attribute this disease to *Pythium* and *Rhizoctonia* (Poletto *et al.*, 2015; Poletto *et al.*, 2007). Studies evaluating the phytosanitary quality of yerba mate seeds have revealed the presence of several fungi, including pathogens such as *Fusarium* (Oliveira *et al.*, 2015; Souza *et al.*, 2019; Souza *et al.*, 2020). Surveys analyzing fungal diseases in yerba mate leaves have also been carried out (Bilenki-Junior *et al.*, 2021; Brito *et al.*, 2021; López *et al.*, 2020; Pimentel *et al.*, 2006), as well as in consumer-ready mate samples (Bernardi *et al.*, 2005; Borges *et al.*, 2002). However, in the case of yerba mate waste, there is still no research on which fungi are associated with it.

In this manner, considering the social-economic importance of this industry to South America and especially considering the way this waste is disposed of in the environment, it is important to know which fungi are associated with yerba mate waste. This can help prevent pathogenic fungi, not only from yerba mate but also from other crops, from being mistakenly discarded into the environment. From this, new control protocols for the use of yerba mate substrate can be provided. Thus, the objective of this work is to evaluate which genera of fungi occur on yerba mate waste.

MATERIAL AND METHODS

Collection of yerba mate residue. The samples were collected during the autumn, winter, and spring seasons of 2020. The collection was carried out for a period of 30 days in each season, being collected by five consumers, totaling 150 samples at the end of each period. The total number of samples analyzed in the experiment was 450. After the consumption of yerba mate in the preparation of *mate* drink, the residue generated was collected in 500 ml canning jars (approximately 160 g of residue per glass). Each glass was partially closed to create an aerobic environment, constituting a humid chamber. There was no addition of water other than the one previously contained in the substrate. The samples were maintained at ambient temperature.

Evaluation of fungi found in yerba mate residue. After 30 days of collection, the samples were evaluated for the occurrence of fungi, and this observation continued for another month. One part of the filamentous fungi, found only on the exposed surface of each glass container, was removed with the aid of tweezers, and a permanent slide was prepared for the microscopic study. This was performed using a Karl Zeiss Axiostar Plus microscope. The fungi were photographed, and pertinent bibliography was used to identify the species found, especially at the genus level using Putzke & Putzke (2008) and specific literature on each genus found. Lists of fungi for yerba mate were prepared by reviewing the literature on the subject.

Statistical analyses. The data were normalized. Afterward, an analysis of variance test (ANOVA) was applied to verify if there was a significant difference between the occurrence and abundance of fungi during the seasons. Then a cluster analysis was applied, with Euclidean distance measure for abundance. Cluster analysis was performed among all seasons and for the abundance and occurrence of fungi within each season.

RESULTS AND DISCUSSION

Twenty-two genera of fungi associated with discarded mate herb residues were found. A literature review on each of the fungal genera found in yerba mate residue was conducted, in order to indicate which associations of these genera have been previously described for yerba mate (Table 1).

Table 1. Filamentous fungi found in yerba mate (*Ilex paraguariensis* St. Hil) residuals.

Species	Kind of relationship	Reference
<i>Allomyces</i> sp.	Saprophytic. First occurrence in association with <i>Ilex paraguariensis</i> .	
<i>Alternaria</i> sp.	Found on seeds	Palmucci <i>et al.</i> (2011)
<i>Arthrotrypis</i> sp.	Spores found on flowers and fruits	Souza <i>et al.</i> (2019)
<i>Aspergillus</i> sp.	Found on seeds, saprophytic and comaninanging of processed mate.	Lin <i>et al.</i> (2018)
<i>Aureobasidium</i> sp.	Saprophytic and pathogenic	Bergottini <i>et al.</i> (2017)
<i>Bipolaris</i> sp.	Found on seeds	Palmucci <i>et al.</i> (2011); Thines & Choi (2016)
<i>Cercospora</i> sp.	Pathogenic of twigs	Oliveira <i>et al.</i> (2015)
<i>Chaetomium</i> sp.	Found on seeds, saprophytic	Lin <i>et al.</i> (2018)
<i>Colletotrichum</i> sp.	Pathogenic causing anthracnose and black-paint	Souza <i>et al.</i> (2019); Dixon (2021)
<i>Curvularia</i> sp.	On seeds, phytopathogenic	Lin <i>et al.</i> (2018)
<i>Fusarium</i> sp.	Pathogenic of leaves, twigs and seeds	Lin <i>et al.</i> (2018)
<i>Mortierella</i> sp.	Saprophytic. Symbiotic with roots	Poletto <i>et al.</i> (2015)
<i>Mucor</i> sp.	Saprophytic. Contaminating of processed mate	Lin <i>et al.</i> (2018)
<i>Nigrospora</i> sp.	Found on seeds	Souza <i>et al.</i> (2019)
<i>Penicillium</i> sp.	Saprophytic. On seeds and contaminating processed mate	Lin <i>et al.</i> (2018)
<i>Peronospora</i> sp.	Pathogenic. First occurrence in association with <i>Ilex paraguariensis</i> .	Paula <i>et al.</i> (2018)
<i>Pilobolus</i> sp.	Saprophytic. First occurrence in association with <i>Ilex paraguariensis</i> .	-
<i>Pythium</i> sp.	Pathogenic. Associated to root rot.	Poletto <i>et al.</i> (2015)
<i>Rhizoctonia</i> sp.	Pathogenic	Lin <i>et al.</i> (2018)
<i>Rhizopus</i> sp.	Saprophytic. Found in seeds and processed mate	Lin <i>et al.</i> (2018)
<i>Thanatephorus</i> sp.	Pathogenic to roots	Dixon (2021)
<i>Verticillium</i> sp.	Saprophytic	Souza <i>et al.</i> (2019)

The genera initially present in the residue consisted of the Zygomycota molds, specifically *Rhizopus* spp. and *Mucor* spp., which were found to be the primary colonizers and dominant for at least 19 days during the experiment, covering 100% of the exposed surface. These species are non-pathogenic and are mainly associated with fruit rots (Poletto *et al.*, 2015). Subsequently, other filamentous fungi, particularly

pathogenic species from the genera *Alternaria*, *Curvularia*, *Fusarium*, *Verticillium*, and *Pythium* (Poletto *et al.*, 2015), begin to appear, sometimes still in association with Zygomycota, but in a final stage with reduced mycelial coverage. The initial colonization of *Mucor* and *Rhizopus* in the substrate was also observed in studies of fungal succession in litter decomposition, where they were considered as the primary colonizers, while late colonizers such as *Aspergillus*, *Penicillium*, and *Chaetomium* were found in other studies of fungal succession in yerba mate (Kumar *et al.*, 2020).

The last stage of fungal succession in this waste leads to a fully darkened and highly moist residue, indicating the dominance of yeast-like fungi and bacteria, and is characterized by the strong odor emanating from some samples in the final stages of the evaluation. This phase can be termed as the maturation phase, where an increase in bacterial proliferation occurs, leading to a decline in the fungal population, possibly due to microbial antagonisms, antibiosis, high moisture content, and slightly acidic pH of the compound (Zhou *et al.*, 2016). This finding is consistent with other observations of an "obvious succession" of fungal communities during the decomposition process, with the filamentous fungi appearing in the initial stages (Tian *et al.*, 2017).

It is noteworthy that the proliferation of insects and nematodes in the residual material was observed in some glass containers and on microscope slides, limiting the development of filamentous fungi as they can serve as food source on them, reducing the observation time, which is one of the indicators that the substrate is reaching its maturation stage (Steel *et al.*, 2018). Nematodes belonging to the genus *Meloidogyne* spp. are mainly associated with yerba mate seedlings, causing root infestations (galling) and problems with growth, yellowing, wilt, and drought. The root-knot nematode (*Meloidogyne incognita*) is considered one of the main phytopathogens of the yerba mate, causing economic losses of up to 56% in some plantations (Laurindo & Souza, 2020).

Given that our study observed 12 genera of fungal known to be pathogenic to yerba mate, all of which are generalists capable of parasitizing other plants, it is important to consider that directly using the residue from yerba mate consumption as an organic fertilizer in flower beds, fruit trees or vegetable gardens - a common practice among the general population- is completely misguided (Santos *et al.*, 2019). This practice introduces these viable fungi, as demonstrated here, into new environments and increases the risk of symptom reproduction in new plants, as these fungi have a broad host range. *Fusarium oxysporum*, for example, is one of the most economically important pathogenic fungi globally, causing root, stem, and fruit rot in numerous cultivated species, resulting in production losses equivalent to one billion dollars (Rampersad, 2020).

New pathogenic fungi of yerba mate are constantly found, as in the case of *Ceratocystis fimbriata* Ellis & Halst in two southern Brazilian municipalities, located 163 km apart, causing losses of 5% in plants of different ages (Santos *et al.*, 2018). In Argentina, the species *Ceratobasidium niltonsouzanum* MP Melo, SI Moreira & PC Ceresini 2018 was identified as pathogenic to yerba mate for the first time, causing symptoms such as white leaf rust and mycelium growth on young branches, indicating that the diversity associated with yerba mate still needs further studies (Lima *et al.*, 2019).

An urgent concern arises regarding the proper disposal of yerba mate residues, particularly due to the presence of many viable pathogens, as highlighted in the present study. Even after undergoing heat treatments in farming, industry, and the domestic settings, reproduction structures of pathogens persist, and their deposition in the environment increases the inoculum potential (Silva *et al.*, 2019; Dors, 2017; Medeiros *et al.*, 2019). This can lead to the contamination of new hosts, the capacity to infect other areas of yerba mate production, and the reduction in the production of this important source of income in southern Brazil. The reintroduction of industrial yerba mate residue to replace the exported macronutrients (N, P, and K), for example, was evaluated and considered as an option as no adverse effects on the soil and plant were observed, although the presence of phytopathogenic fungi was not evaluated (Sousa *et al.*, 2015).

When the analysis of variance (ANOVA) was conducted to determine whether there was a significant difference in the occurrence and abundance of fungi among seasons, no significant difference was observed. However, when the clusters were evaluated based on the occurrence and abundance of fungi (using Euclidean distance), it was observed that the fungi found in autumn and spring seasons had a greater proximity in terms of occurrence and abundance (Figure 1).

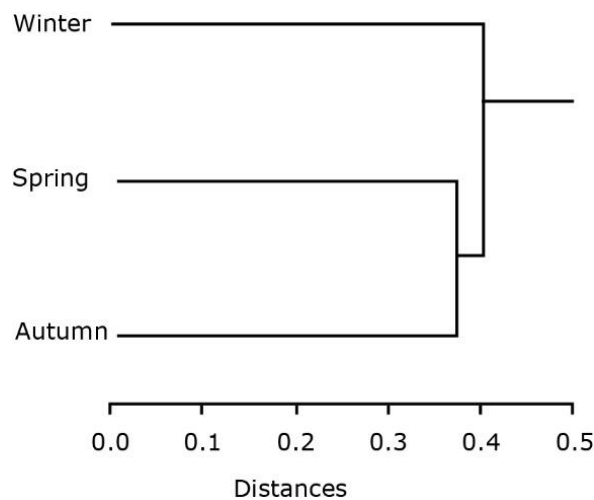


Figure 1: Cluster analysis showing the relationship of abundance and occurrence of fungi found at the three seasons analyzed.

Among the twenty-two genera of fungi found associated with yerba mate, according to literature review, twelve of them are considered pathogenic not only to yerba mate but also to several other commercial crops. This study contributes to the expansion of knowledge regarding the fungi associated with *Ilex paraguariensis*, highlighting that the pathogens remain viable in the final waste, even after all the processing and use by the consumer.

Therefore, greater attention is required when disposing the yerba mate byproducts. Alternatives such as separate collection of organic waste generated in the producing regions, proper disposal in landfills, use of the substrate for growing edible mushrooms, or some other additional form of sterilization that still needs evaluation are mentioned here as possible solutions.

CONCLUSIONS

This study contributes to the expansion of our understanding of the fungal communities associated with *I. paraguariensis*, particularly with regards to the waste generated by the consumption of yerba mate. The presence of numerous fungal genera, known to be pathogenic according to literature, highlights the importance of proper waste disposal practices in the environment. Given that the improper disposal of this waste can lead to contamination of not only yerba mate but also other commercial crops, it is imperative that greater attention be paid to its disposal.

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