

## EFFECT OF *Ilex paraguariensis* St. Hil. AND *Coffea arabica* L. ON THE GROWTH OF *Fonsecaea pedrosoi* ATCC 46428

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### ABSTRACT

This work evaluated the effect of aqueous extracts from *Ilex paraguariensis* (maté) and *Coffea arabica* (coffee) combined with Sabouraud dextrose agar on the growth of *Fonsecaea pedrosoi* ATCC 46428. *F. pedrosoi* was grown on Petri dishes containing Sabouraud dextrose agar amended with aqueous extract derived from 0.5; 1; 2; 3; 4 and 5g of maté or coffee powder boiled in 100ml of water for 30 min. The diameters of fungal colonies were determined after 7 days. The incorporation of maté or coffee extracts into the growth media did not cause significant differences in the radial growth of *F. pedrosoi* ATCC 46428 when compared to the control. Nevertheless, nutritional requirement studies are important to the systematization of the biochemical profile, which may contribute to elucidating the functional biochemistry of *F. pedrosoi*.

**Key-words:** *Fonsecaea pedrosoi*. *Ilex paraguariensis*. *Coffea arabica*. aqueous extract

### 1 Introduction

*Fonsecaea pedrosoi* is a dematiaceous fungus which, among other pathogens such as *Phialophora verrucosa*, *Cladosporium carrionii*, *Rhinochrysiella aquaspersa* and *Fonsecaea compactum* [1-2], causes a chronic subcutaneous disease in man, rarely in animals, called chromoblastomycosis [3-4]. Infection begins with the traumatic implantation of conidia or hyphal fragments into subcutaneous tissues, producing initial lesions consisting of papules or nodules that become verrucous [5]. The disease occurs mainly among male agricultural laborers and primarily affects the lower limbs [6-9]. Current therapies against chromoblastomycosis involve the use of antifungal agents and/or surgical excision but, as in other subcutaneous mycoses, the treatment is poorly effective, producing relapses during therapy and problems with lack of tolerance to antifungal drugs [10-11].

The caffeine (1,3,7-trimethylxanthine) is an alkaloid found in more than 60 plant species. Caffeine is present at significant levels in coffee, tea, cocoa and in different types of cola drinks and is considered toxic to many microorganisms; however, some microorganisms have the ability to grow in the presence of caffeine and the capacity to degrade the alkaloid [12]. Several studies were carried out to investigate the use of caffeine as a source of energy for microbial growth [13-14]. On the other hand, some activities as chitinase inhibition [15-16] and fungistasis [17] have been related to the presence of methylxanthines, caffeine, and theobromine [18]. *Penicillium* and

*Aspergillus* are the most frequent caffeine-degrading fungal genera, whereas *Pseudomonas* is the genus that most often degrades bacteria [19-20].

The aim of this work was to evaluate and compare the effect of aqueous extracts from *Ilex paraguariensis* St. Hil. or *Coffea arabica* L. combined with Sabouraud dextrose agar on the growth of *Fonsecaea pedrosoi* ATCC 46428.

### 2 Materials and Methods

**Strain:** *F. pedrosoi* ATCC 46428 was purchased from the Laboratory of Pathogenic Fungi, Institute of Basic Health Sciences, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.

**Pre-inoculum:** The strain was grown on Petri dishes containing Sabouraud dextrose agar (SDA) at 37°C for 21 days to ensure full cover of the media.

**Extracts:** each 0.5; 1; 2; 3; 4 and 5g of commercially obtained maté powder and coffee powder was boiled in 100ml of distilled water for 30 minutes. 20ml from each filtrate was added to 1.3g of SDA, sterilized and poured onto plates. For comparison, the same quantity of distilled water was mixed into the SDA media and sterilized.

**Test:** three culture discs were inoculated onto each medium for 7 days at 25°C. The colony diameter was determined with a caliper and compared to the control (SDA only).

### 3 Results and Discussion

To define media for the growth of *F. pedrosoi*, experiments were performed to verify the behavior of this microorganism grown at different concentrations of maté and coffee powder extracts. The incorporation of *I. paraguariensis* and *C. arabica* extracts into the growth media did not cause significant differences in the diameters of the fungal strain when compared to the control, as shown in Figure 1.

Caffeine and theobromine concentrations vary in *Ilex* and *Coffea* species according to the extraction method. Reginatto et al. [21] obtained, from chloroform:methanol extraction of maté leaves, about 0.65% of caffeine and 0.12% of theobromine/dry weight. Aqueous extracts can yield similar values, about 0.67% of caffeine and 0.07% of theobromine/dry weight [18].

Caffeine in coffee beans is influenced by genetic and environmental factors, but its concentration can achieve 1.2% on *var. arabica* and 2.0% on *var. robusta* [22]. It is not only caffeine that has some effect on fungal growth; depending on the rate of mixture of varieties, year and degree of roast, distinct quantities of proteins, sugars and polyphenols can be found [23].

Methylxanthines can be mutagenic through inhibition of DNA repair in bacteria [24-25] and, at 0.1% or higher, they can also inhibit the growth of different filamentous fungi [17]. However, some microorganisms have the ability to grow in the presence of caffeine and show capacity to degrade these alkaloids [12].

We conclude that nutritional requirement studies are important to the systematization of the biochemical profile, which may contribute to elucidating the functional biochemistry of *F. pedrosoi*.

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### EFEITO DE *Ilex paraguariensis* St. Hil. e *Coffea arabica* L. SOBRE O CRESCIMENTO DE *Fonsecaea pedrosoi* ATCC 46428

#### Resumo

Este trabalho avaliou o efeito de extratos aquosos de *Ilex paraguariensis* (erva-mate) e *Coffea arabica* (café) em ágar Sabouraud dextrose no crescimento de *Fonsecaea pedrosoi* ATCC 46428. *F. pedrosoi* foi cultivada em placas de Petri contendo ágar Sabouraud dextrose suplementado com extratos aquosos derivados de 0,5; 1; 2; 3; 4 e 5g de pó de erva-mate ou de café fervidos em 100ml de água destilada por 30 min. Os diâmetros das colônias do fungo foram determinados após 7 dias. A incorporação dos extratos de erva-mate ou café no meio de crescimento não causou diferenças significativas no crescimento radial de *F. pedrosoi* ATCC 46428 comparado ao controle. Entretanto, estudos sobre o requerimento nutricional são importantes na sistematização do perfil bioquímico, o que pode contribuir na elucidação da bioquímica funcional do microorganismo.

**Palavras-chave:** *Fonsecaea pedrosoi*. *Ilex paraguariensis*. *Coffea Arabica*. extrato aquoso

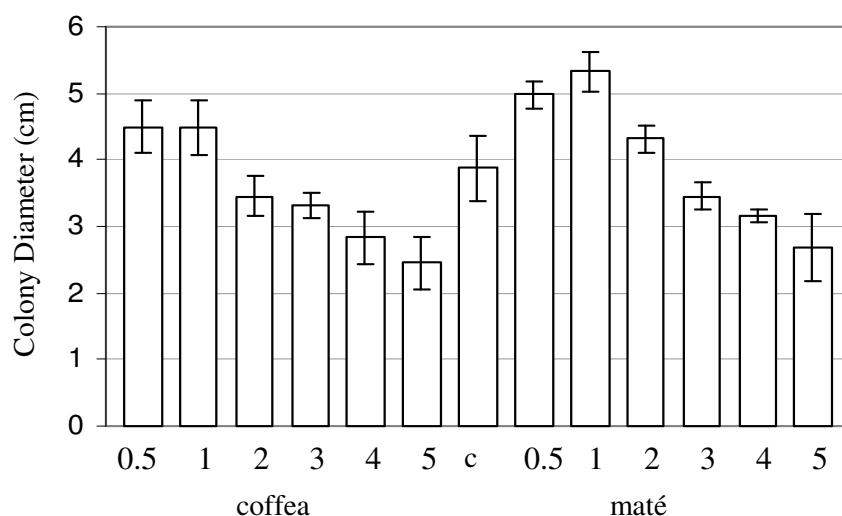


Fig. 1: Colony diameter of *F. pedrosoi* ATCC 46428 grown on Sabouraud dextrose agar amended with aqueous extracts derived from mate and coffee powder (g.100mL<sup>-1</sup>). c= control; error bars: standard deviation. Tests made in triplicate.

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