Eco-epidemiologic study of emerging fungi related to the work of babaçu coconut breakers in the State of Maranhão, Brazil

Maria do Desterro Soares Brandão Nascimento[1],[2], Valéria Maria Sousa Leitão[3], Marcos Antonio Custódio Neto da Silva[4], Leonardo Bezerra Maciel[5], Walbert Edson Muniz Filho[1], Graça Maria de Castro Viana[1] and Geusa Felipa de Barros Bezerra[1]


ABSTRACT

Introduction: There are more than 300,000 extractors using the babaçu coconut as a source of income in the States of Maranhão, Pará, Tocantins and Piauí, and this activity is associated with fungal infections. The objective of this study was to examine the occurrence of emergent fungi in the conjunctiva, nails and surface and subcutaneous injuries of female coconut breakers in Esperantinópolis, Maranhão. Additionally, soil samples and palm structures were collected. Methods: The obtained samples were cultured in Petri dishes containing potato-dextrose-agar and chloramphenicol. The etiological agent was confirmed by a direct mycological exam and growth in culture. Results: In total, 150 domiciles were visited, and samples were collected from 80 patients. From the ground, the most frequently isolated fungus was Aspergillus niger (53.8%). The most frequently detected fungus in babaçu coconut was Aspergillus niger (66.7%). Conjunctival fungal growth occurred in 76.3% of the women. The ocular fungal microbiota consisted of filamentous fungi (80.6%), and yeasts were present in 19.4% of cases. Onychomycosis was diagnosed in 44% (11/25) of the women. Conclusions: The identification of the genera Neosartorya, Rhizopus and Curvularia in onychomycoses shows that emergent filamentous fungi can be isolated. Aspergillus sp., Penicillium sp. and Scedosporium sp. were the predominant genera found in the babaçu coconut. From ocular conjunctiva, Candida spp. were the most prevalent species isolated, and Fusarium sp. was present only in one woman. The nearly permanent exposure of coconut breakers to the external environment and to the soil is most likely the reason for the existence of a mycotic flora and fungal infections, varying according to the individual’s practices and occupation.

Keywords: Fungi. Coconut. Allied Health Occupations.

INTRODUCTION

Mycoses are not diseases of compulsory notification for the World Health Organization. In Brazil, numerous superficial, opportunistic and deep mycoses have been reported, especially in areas where there is a practitioner with professional qualification for handling mycoses.

Due to the considerably increased incidence of fungal infections in humans at various locations around the world, it is becoming important to analyze the frequency of fungal infections, taking into account climatic and geographical variation and, therefore, the origin of the population, in addition to the sex and occupation of the affected individuals.

More than 300,000 extractives use babaçu as a source of income in the States of Maranhão, Pará, Piauí and Tocantins. Babaçu is the main source of income of babaçu coconut breakers. Despite being a natural resource, the product is mostly located in the private domain of large estates with extensive livestock. In Maranhão, the larger formations of babaçu crops are in the lowlands between the coast and the inland plateau, with particular intensity in the valleys of the middle course of the rivers Itapecuru Mearim, Pindaré and Grajaú. Babaçu has important economic value to the Maranhão region, which has an average production of 1.5 metric tons of fruits per hectare.

Babaçu coconut breakers are often affected by diseases, with a seemingly obvious relationship between the type of occupation and the development of fungal infection. The exposure of babaçu coconut breakers to geophilic fungi and phytopathogens predisposes them to fungal infection and mycoses. These problems require clinical and laboratory diagnoses and result in preventive measures, thus justifying the economic and social importance of this labor activity.
This study aimed to identify emerging fungi, the potential agents of mycoses, in the ocular conjunctiva, nails and skin lesions of babaçu coconut breakers in the Zona dos Cocos, Microrregion of Middle Mearim, Maranhão State, municipality of Esperantinópolis, to isolate geophilic fungi and phytopathogens and to relate the extractive activity for the exploitation of the babaçu coconut to superficial and systemic mycoses.

## METHODS

### Study area

Esperantinópolis is located in the Microrregion of Middle Mearim, Meso Center of Maranhão, and has hot and humid weather.

This municipality is located at 4º33’29” S latitude and 44º52’40” W-GR longitude and has an area of 481km² and estimated population of 22,304 inhabitants³.

### Survey of fungi in the soil near the babaçu tree and palm-related structures (coconut shell, coconut, leaf, concavity and cachopa)

Ten samples were collected from the babaçu coconut shell, as well as from its coconut, leaf, concavity and cachopa, using sterile equipment. The samples from the palm were ground, and the fungal colonies were cultivated using the technique of serial suspension, according to Clark⁴. A spoon was introduced in the field at a depth of 5 cm to obtain samples from the soil, which were placed in polyethylene bags and brought to the Laboratory of Basic and Applied Immunology UFMA. The soil was then placed in Petri dishes according to the modified technique of Vanbreuseghem⁵. Subsequently, the colonies were transported to test tubes containing Sabouraud agar and potato agar with chloramphenicol.

After checking the growth of the fungal colonies, direct examination was performed under an optical microscope, and microculture was conducted for taxonomic confirmation.

### Survey of fungi in babaçu coconut breakers (ocular conjunctiva, skin and nails)

In total, 150 domiciles were visited, and samples were collected from 80 patients. These samples corresponded only to individuals who were employed as coconut breakers.

The samples were collected from the inferior fornix of both eyes with sterile swabs and were cultured in Sabouraud agar and potato agar media with chloramphenicol to isolate the fungi.

Additionally, samples were collected from the breakers’ cutaneous and subcutaneous lesions by scraping the skin. Later, the samples were cultured in Sabouraud agar and potato agar media with chloramphenicol to isolate fungi⁶.

Nail samples were obtained from those patients with suspected onychomycosis. Distal subungual samples were obtained by scraping between the normal and affected boundaries of nails and hyperkeratotic nail beds. In proximal onychomycosis, a stylus was used for deeper collection. Each sample was studied by direct microscopic examination after clarification for 30min in 20% sodium hydroxide. The nail scrapings were inoculated on Sabouraud agar and potato agar with chloramphenicol⁷.

The identification of the etiologic agent was based on studying the macroscopic and microscopic aspects of the colonies.

### Statistical analysis

The data were computerized using Biostatic Software 4.0, which was also used to perform the subsequent statistical analysis.

### Ethical considerations

This project was approved by the Research Ethics Committee of the University Hospital-UFMA (number 287/06).

### RESULTS

In total, 13, 4, 12, 7, 10 and 7 fungal isolates were obtained from the soil samples collected near babaçu trees, the babaçu coconut shell samples, the babaçu coconut samples, the palm tree leaf samples, the palm cachopa samples and the palm concavity samples, respectively. The macro- and micromorphological characteristics of the colonies led to the diagnoses (Table 1).

A total of 25 samples from nails with changes in their morphology that were suggestive of onychomycosis were collected. Of the 25 cultured samples, 14 cultures were negative (56%), and 11 were positive (44%) (Table 2).

All four skin samples from the babaçu coconut breakers that were collected and examined were consistent with what was suggested in the clinical diagnosis, i.e., *Malassezia furfur*.

From the ocular conjunctiva of the 80 babaçu coconut breakers who were evaluated, 76.3% (64) of cultures were positive for fungi (Table 2). In total, 72 fungal isolates were collected from the ocular conjunctiva. The fungi that were most frequently found in the ocular conjunctiva were filamentous fungi, affecting 58 (80.5%) breakers, and yeasts corresponding to *Candida* sp. and *Malassezia* sp. were found in 14 (19.4%) breakers.

In an analysis of the presence of isolated fungi and the collection site in babaçu coconut breakers, there was not a significant difference (p = 0.22) (Table 3).

### DISCUSSION

Soil is considered to be one of the main habitats of the microbial population, which includes fungi⁸. In soil, fungi are found in communities ranging from 10⁷ to 10⁹ organisms per gram⁹ that actively participate in the processes of biodeterioration and biodegradation¹⁰, contributing to nutrient cycling and, thus, the maintenance of ecosystems.

Phytopathogenic fungi inhabiting the soil are a problem that is difficult to control in a cultivated area, particularly once they produce resistance structures that can survive in the soil for several years. The species found in this study

<table>
<thead>
<tr>
<th>Variables</th>
<th>Fungi isolation from soil</th>
<th>Fungi isolation from coconut shell</th>
<th>Fungi isolation from coconut</th>
<th>Fungi isolation from palm leaf</th>
<th>Fungi isolation from cachopa</th>
<th>Fungi isolation from concavity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aspergillus niger</td>
<td>7 (7/13) 53.8</td>
<td>2 (2/4) 50.0</td>
<td>6 (6/7) 85.7</td>
<td>2 (2/7) 28.6</td>
<td>2 (2/7) 28.6</td>
</tr>
<tr>
<td></td>
<td>Aspergillus nidulans</td>
<td>1 (1/13) 7.7</td>
<td>2 (2/4) 50.0</td>
<td>1 (1/7) 14.3</td>
<td>2 (2/7) 28.6</td>
<td>2 (2/7) 28.6</td>
</tr>
<tr>
<td></td>
<td>Penicillium sp.</td>
<td>2 (2/13) 15.4</td>
<td>1 (1/13) 7.7</td>
<td>2 (2/10) 20.0</td>
<td>2 (2/7) 28.6</td>
<td>2 (2/7) 28.6</td>
</tr>
<tr>
<td></td>
<td>Scedosporium sp.</td>
<td>2 (2/13) 15.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Syncephalastrum sp.</td>
<td>1 (1/13) 7.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Variables</th>
<th>Fungi isolated from nails</th>
<th>Presence of fungi in ocular conjunctiva</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>yeasts</td>
<td>positive</td>
</tr>
<tr>
<td></td>
<td>Neosartorya spinosa</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>Trichophyton sp.</td>
<td>unknown</td>
</tr>
<tr>
<td></td>
<td>Rhizopus sp.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Curvularia sp.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aspergillus niger</td>
<td>4 (4/25) 36.4</td>
</tr>
<tr>
<td></td>
<td>Penicillium sp.</td>
<td>2 (2/25) 18.2</td>
</tr>
<tr>
<td></td>
<td>yeasts</td>
<td>2 (2/25) 18.2</td>
</tr>
<tr>
<td></td>
<td>Aspergillus niger</td>
<td>11 (11/80) 15.3</td>
</tr>
<tr>
<td></td>
<td>Candida sp.</td>
<td>11 (11/80) 15.3</td>
</tr>
<tr>
<td></td>
<td>Penicillium sp.</td>
<td>07 (07/80) 9.7</td>
</tr>
<tr>
<td></td>
<td>Syncephalastrum sp.</td>
<td>03 (03/80) 4.2</td>
</tr>
<tr>
<td></td>
<td>Nigrospora sp.</td>
<td>03 (03/80) 4.2</td>
</tr>
<tr>
<td></td>
<td>Malassezia sp.</td>
<td>03 (03/80) 4.2</td>
</tr>
<tr>
<td></td>
<td>Sporothrix sp.</td>
<td>02 (02/80) 2.7</td>
</tr>
<tr>
<td></td>
<td>Cladosporium sp.</td>
<td>02 (02/80) 2.7</td>
</tr>
<tr>
<td></td>
<td>Aspergillus versicolor</td>
<td>01 (01/80) 1.4</td>
</tr>
<tr>
<td></td>
<td>Aspergillus flavus</td>
<td>01 (01/80) 1.4</td>
</tr>
<tr>
<td></td>
<td>Aspergillus nidulans</td>
<td>01 (01/80) 1.4</td>
</tr>
<tr>
<td></td>
<td>Cladophialophora sp.</td>
<td>01 (01/80) 1.4</td>
</tr>
<tr>
<td></td>
<td>Trichophyton sp.</td>
<td>01 (01/80) 1.4</td>
</tr>
<tr>
<td></td>
<td>Fusarium sp.</td>
<td>01 (01/80) 1.4</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Fungi</th>
<th>Yeasts</th>
<th>Trichophyton sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site in women</td>
<td>F</td>
<td>%</td>
</tr>
<tr>
<td>Conjunctiva</td>
<td>11</td>
<td>15.27 (11/80)</td>
</tr>
<tr>
<td>Skin/nails</td>
<td>4</td>
<td>36.4 (4/25)</td>
</tr>
</tbody>
</table>

\( \chi^2 = 1.8; p = 0.22 \)

F: frequency; \( \chi^2 \): chi-square.
Most authors have frequently identified dermatophytes as agents (80 to 90%), followed by yeasts (5 to 17%) and non-dermatophytic filamentous fungi (2 to 12%)\textsuperscript{17,18}, in agreement with the present study.

In this study, \textit{Neosartorya spinosa}, \textit{Trichophyton} sp., \textit{Rhizopus} sp. and \textit{Curvularia} sp. were isolated from nails with suspected onychomycosis, in agreement with the findings of other authors. A higher frequency of yeasts was also demonstrated in a study in which \textit{Candida} sp. were more common in fingernails and women, and this observation has also been shown by other researchers\textsuperscript{19}.

Regarding the fungi isolated from the ocular conjunctiva, \textit{Aspergillus} was predominant. In agreement with this study, in decreasing order of frequency, the following fungi have been isolated from the conjunctiva: \textit{Aspergillus} sp., \textit{Candida}, \textit{Penicillium}, \textit{Syncephalastrum}, \textit{Nigrospora}, \textit{Malassezia}, \textit{Sporothrix}, \textit{Cladosporium}, \textit{Cladophialophora}, \textit{Trichophyton} and \textit{Fusarium}. \textit{Aspergillus} sp. and yeasts are the most commonly isolated genera in most reports on the mycobiota in healthy conjunctiva, mycotic keratitis and anemophilous microbiota\textsuperscript{20-24}.

Among various studies, the different percentages of fungi isolated from the normal conjunctival microbiota are related to many factors, such as the different personal and general hygienic conditions of the population evaluated, the different climatic conditions and vegetation of the region studied and whether the region was urban or rural\textsuperscript{25}. Additionally, the influence of socioeconomic patterns was shown by Olson, who found an 85% incidence of fungi in individuals of lower socioeconomic levels and a 36% incidence in individuals of higher socioeconomic levels\textsuperscript{26}.

In Brazil, the frequency of fungi isolated from the conjunctiva of healthy individuals varies from 0\textsuperscript{27} to 72%\textsuperscript{17}. The fungi most frequently isolated from these eyes are \textit{Aspergillus} sp., \textit{Candida} sp., \textit{Penicillium} sp., \textit{Cladosporium} sp. and \textit{Alternaria} sp.\textsuperscript{24,25}.

In the environment, \textit{Penicillium} sp. is the second most isolated fungus\textsuperscript{21}, indicating that the environment can contaminate conjunctiva transiently. Thus, the high isolation frequency of this fungus in the present study may have been due to environmental contamination\textsuperscript{28}.

The nearly permanent exposure of the conjunctiva to the external environment is perhaps the most logical reason for the existence of a mycotic flora in healthy conjunctiva, varying according to the individual’s merits and occupation\textsuperscript{24-30,33}. In most cases, babaçu coconut breakers perform their activities without personal protective equipment, which exposes them to the risks inherent to the work, as several of them perform this activity when working with babaçu crops. This practice allows contamination of the eyeball by anemophilous microbiota and leaves.

The isolates from the coconut breakers were similar to those found in the palm study. The nearly permanent exposure of coconut breakers to the external environment and soil is perhaps the most logical reason for the existence of the mycotic flora and fungal infections, varying according to the individual’s merits and occupation.

### ACKNOWLEDGMENTS

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### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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