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

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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[1].

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[2], in addition to the sex and occupation of the affected individuals[3].

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[4]. 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[5].

Babaçu coconut breakers are often affected by diseases, with a seemingly obvious relationship between the type of occupation and the development of fungal infection[6]. 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[4].

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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 Cocais, Microregion 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 Microregion 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 481km^2 and estimated population of 22,304 inhabitants[7].

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 Clark[8]. 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 Vanbreuseghem[9]. 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 fungi[1].

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 chloramphenicol[1].

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 fungi[10]. In soil, fungi are found in communities ranging from 10^4 to 10^6 organisms per gram[11] that actively participate in the processes of biodeterioration and biodegradation[12], 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 1 - Distribution and taxonomic classification of fungi isolated from soil near babaçu trees and from the babaçu coconut shell, coconut, leaf, cachopa and palm concavity. Esperantinópolis, State of Maranhão, 2007.

Variables	n	%
Fungi isolation from soil		
Aspergillus niger	7 (7/13)	53.8
Aspergillus nidulans	1 (1/13)	7.7
Penicillium sp.	2 (2/13)	15.4
Scedosporium sp.	2 (2/13)	15.4
Syncephalastrum sp.	1 (1/13)	7.7
Fungi isolation from coconut shell		
Aspergillus niger	2 (2/4)	50.0
Penicillium sp.	2 (2/4)	50.0
Fungi isolation from coconut		
Aspergillus niger	8 (8/12)	66.7
Aspergillus versicolor	2 (2/12)	16.7
Aspergillus flavus	1 (1/12)	8.3
Penicillium sp.	1 (1/12)	8.3
Fungi isolation from palm leaf		
Aspergillus niger	6 (6/7)	85.7
Penicillium sp.	1 (1/7)	14.3
Fungi isolation from cachopa		
Aspergillus niger	6 (6/10)	60.0
Penicillium sp.	2 (2/10)	20.0
Scedosporium sp.	2 (2/10)	20.0
Fungi isolation from concavity		
Aspergillus niger	2 (2/7)	28.6
Penicillium sp.	2 (2/7)	28.6
Acremonium sp.	1 (1/7)	14.3
Cladosporium sp.	1 (1/7)	14.3
Chaetomium sp.	1 (1/7)	14.3
Total	53	100.0

TABLE 2 - Distribution and taxonomic classification of fungi isolated from the nails and ocular conjunctiva of babaçu coconut breakers and control groups. Esperantinópolis, State of Maranhão, 2007.

Variables	n	%
Fungi isolated from nails		
yeasts	4 (4/25)	36.4
Neosartorya spinosa	2 (2/25)	18.2
Trichophyton sp.	2 (2/25)	18.2
Rhizopus sp.	2 (2/25)	18.2
Curvularia sp.	1 (1/25)	9.0
Presence of fungi in ocular conjunctiva		
positive	61 (61/80)	76.3
negative	16 (16/80)	20.0
unknown	3 (3/80)	3.7
Fungi isolated from ocular conjunctiva		
Aspergillus sp.	24 (24/80)	33.3
Aspergillus niger	11 (11/80)	15.3
Candida sp.	11 (11/80)	15.3
Penicillium sp.	07 (07/80)	9.7
Syncephalastrum sp.	03 (03/80)	4.2
Nigrospora sp.	03 (03/80)	4.2
Malassezia sp.	03 (03/80)	4.2
Sporothrix sp.	02 (02/80)	2.7
Cladosporium sp.	02 (02/80)	2.7
Aspergillus versicolor	01 (01/80)	1.4
Aspergillus flavus	01 (01/80)	1.4
Aspergillus nidulans	01 (01/80)	1.4
Cladophialophora sp.	01 (01/80)	1.4
Trichophyton sp.	01 (01/80)	1.4
Fusarium sp.	01 (01/80)	1.4

(*Aspergillus niger, Aspergillus versicolor, Aspergillus flavus, Aspergillus nidulans, Penicillium, Scedosporium, Acremonium, Cladosporium, Chaetomium* and *Syncephalastrum*) were isolated from the ground[13-15].

The fungi found in the soil, stems, bark, leaves, coconuts, cachopa and concavity are in agreement with the filamentous fungi isolated from soil in municipalities in the region of Xingó, Brazil[16].

In the analysis of the fungi isolated from the nail samples of babaçu coconut breakers, filamentous fungi were predominant (63.6%).

TABLE 3 - Frequencies of fungal isolates and their relationship with the body part. Esperantinópolis, State of Maranhão, 2007.

Fungi	Yeasts		*Trichophyton* sp.	
Site in women	F	%	F	%
Conjunctiva	11	15.27 (11/80)	1	1.38 (1/80)
Skin/nails	4	36.4 (4/25)	2	18.2 (2/25)
		$X^2 = 1.8$; $p = 0.22$		

F: frequency; X^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%)[17,18], in agreement with the present study.

In this study, *Neosartorya spinosa*, *Trichophyton* sp., *Rhizopus* sp. and *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 *Candida* sp. were more common in fingernails and women, and this observation has also been shown by other researchers[19].

Regarding the fungi isolated from the ocular conjunctiva, *Aspergillus* was predominant. In agreement with this study, in decreasing order of frequency, the following fungi have been isolated from the conjunctiva: *Aspergillus* sp*., Candida, Penicillium, Syncephalastrum, Nigrospora, Malassezia, Sporothrix, Cladosporium, Cladophialophora, Trichophyton* and *Fusarium*. *Aspergillus* sp. and yeasts are the most commonly isolated genera in most reports on the mycobiota in healthy conjunctiva, mycotic keratitis and anemophilous microbiota[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[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[26].

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

In the environment, *Penicillium* sp. is the second most isolated fungus[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[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[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.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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REFERENCES

1. Lacaz CS, Poero E, Martins JEC. Tratado de Micologia Médica. 9th. ed. São Paulo: Sarvier; 2002.
2. Godoy PC. Diversidade genotípica de cepas de *Fusarium solani* isoladas de episódios de ceratites [Doctors Thesis] [São Paulo]: Universidade Federal de São Paulo, Escola Paulista de Medicina; 2004.
3. Salera CM, Tanure MAG, Lima WTM, Campos CM, Trindade FC, Moreira JA. Perfil das ceratites fúngicas no Hospital São Geraldo Belo Horizonte – MG. Arq Bras Oftalmol 2002; 65:9-13.
4. Associação em Áreas de Assentamentos no Estado do Maranhão (Assema). Programa de Organização de Mulheres**.** São Luis-MA. [Cited 2007 August 22]. Available at: http://www.assema.org.br/geral.php/
5. Cardoso MF. O Maranhão por Dentro. São Luis: Lithograf; 2001.
6. Silva CMP, Rocha RM, Moreno JS, Branco MRFC, Silva RR, Marques SG, et al. O babaçu (*Orbignya phalerata martins*) como provável fator de risco de infecção humana pelo agente da cromoblastomicose no Estado do Maranhão. Rev Soc Bras Med Trop 1995; 28:49-52.
7. Instituto Brasileiro De Geografia e Estatística (IBGE). Cidades. [Cited 2007 October 10]. Available at: http://www.ibge.g.,ov.br/cidadesat/painel/painel.php?codmun=210300#/.
8. Clark FE. Agar-plate method for total microbial count. *In*: Blanc CA, Evans D, White JL, Ensminger LE, Clark FE, Dinauer RC, editors. Methods of soil analysis. Chemical and microbiological properties. Part II.. New York: Madson Inc; 1965; p.1460-1466.
9. Vanbreuseghem R. Technique biologique pour I isolement des dermatophytes du sol. Ann Soc Belge Med Trop 1952; 32:173-178.
10. Paul EA, Clark FE. Soil Microbiology and Biochemistry. San Diego: Academic Press; 1989.
11. Alexander M. Introduction to Soil Microbiology. New York: John Weley; 1977.
12. Eggins HO, Allsop D. Biodeterioration and biodegradation by fungi. *In*: Smith JE, Berry DR, editors. Industrial Mycology. The filamentous fungi. Vol. 1. London: Edward Arnold; 1975, p 301-19.
13. Domsch KH, Gams W, Anderson TH. Compendium of soil fungi. Vol 1. San Francisco: Academic Press; 1993.
14. Ellis MB. Dematiaceus Hyphomycetes. Kew: Commomwealth Mycologi Institute; 1971.
15. Raper KB, Fenell DI. The genus *Aspergillus*. New York: Robert and Krieger Malabar Publishing Company; 1977.

16. Cavalcanti MAQ, Oliveira LG, Fernandes MJ, Lima DM. Fungos filamentosos isolados do solo em municípios na região Xingó, Brasil. Acta Bot. Bras 2006; 20:831-837.

17. Gupta AK, Konnikov N, MacDonald P, Rich P, Rodger NW, Edmonds MW, et al. Prevalence and epidemiology of toenail onychomycosis in diabetic subjects: a multicentre survey. Br J Dermatol 1998; 139:665-671.

18. Jaffe R. Onychomycosis: recognition, diagnosis, and management. Arch Fam Med 1998; 7:587-592.

19. Greer DL. Evolving role of nondermatophytes in onychomycosis. Int J Dermatol 1995; 34:521-524.

20. Ando N, Takatori K. Fungal flora of the conjunctival sac. Am J Ophthalmol 1982; 94:67-74.

21. Scarpi MJ, Belfort JR, Gompeltz OF. Microbiota fúngica da conjuntiva normal de trabalhadores no corte de cana-de-açúcar. Rev Bras Oftalmol 1985; 44:57-65.

22. Mitsui Y, Hanabusa J. Corneal infections after cortisone therapy. Br J Ophthalmol 1955; 39:244-250.

23. Tomar VPS, Sharma OP, Joshi K: Bacterial and fungal flora of normal conjunctiva. Ann Ophthalmol 1971; 3:669-671.

24. Sehgal SC, Dhawan S, Chhiber S, Sharma M, Talwar P. Frequency and significance of fungal isolations from conjunctival sac and their role in ocular infections. Mycopathologia 1981; 73:17-19.

25. Cha SB, Fischman O, Barros PSM, Mikoves R. Microbiota fúngica conjuntival de pacientes com síndrome da imunodeficiência adquirida (AIDS). Arq Bras Oftalmol 1990; 53:80-90.

26. Olson CL. Fungal contamination of conjunctiva and lid margin. Arch Ophthalmol 1969; 81:351-355.

27. Azevedo ML. Investigações preliminares sobre a microbiota ocular. Arq Bras Oftalmol 1962; 25:41-47.

28. Rosa RH, Miller D, Alfonso EC. The changing spectrum of fungal keratitis in South Florida. Ophthalmology 1994; 101:1005-1113.

29. Ainley R, Smith B. Fungal flora of the conjunctival sac in healthy and diseased eyes. Br J Ophthalmol 1965; 49:505-515.

30. Andrade AJM, Hofling-Lima AL, Yu MCZ, Godoy P, Gompertz OF, Bonfim SS, Andrade FEM. Estudo da microbiota em conjuntiva sadia de diabéticos, residentes na área urbana da cidade de São Paulo - Brasil. Arq Bras Oftalmol 2006; 69:75-83.

31. Santos PM, Melo FSM, Melo CM, Maltez LCGM, Santos RCR. Estudo comparativo da flora fúngica conjuntival em portadores de hanseníase de Hospital-Colônia e nos indivíduos sadios no Planalto Central. Rev Bras Oftalmol 2004; 63:533-537.

32. Santos PM, Muccioli C, Santos RCR, Martins SAR, Fishman O, Belfort Júnior R. Microbiota fúngica conjuntival: estudo comparativo entre pacientes com AIDS, pacientes infectados pelo HIV e pacientes HIV-negativos antes da era do HAART. Arq Bras Oftalmol 1999; 62: 731-734.

33. Costa ML, Galvão PG, Lage J. Flora micótica da conjuntiva de indivíduos normais. Rev Bras Oftalmol 1975; 34:199-206.