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Original Article

Isolation of Dematiaceous Fungi from Soil and their Pathogenic Potentials.

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ABSTRACT

The presence of dematiaceous fungi in 200 samples of soil collected from 4 different villages located in South-Eastern Nigeria were investigated. Using the pour plate method, the samples where cultured in Saboraud dextrose agar with 0.05mg/ml of chloramphenicol and incubated at room temperature. Identification of isolates was by slide culture technique and the following dematiaceous fungi were identified at different proportions; *Madurella grisea* 20 (43%), *Fonsecaea dermatitidis* 10 (21.7%), *Cladosporium werneckii* 10 (21.7%), *Madurella mycetomi* 5(10.8%), and *Leptosphaeria senegalensis* 1 (2.17%). Loamy soil yielded the highest number of varients of dematiaceous fungi. *Madurella grisea* and *Fonsecaea dermatitidis* were pathogenic for laboratory mice when injected subcutaneously. The findings of several dematiaceous fungi in soil samples in these villages may represent possible pattern of infections in humans since people residing in the study area were rural farmers.

Keywords: Mycoses; Soil; Infection.

INTRODUCTION

The demonstration of saprophytic existence of pathogenic and non pathogenic fungi in a particular region serves as reliable index of endemicity of infections caused by them. Soil that serves as home for many organisms also serve as reservoir of infections they cause1,2 Dematiaceous fungi are heterogeneous group darkly pigmented fungi widely distributed in the environment that occasionally cause infections in humans, animals and plants3. When grown on agar, colonies are dark grey, brown and importantly have a black reverse when the bottom of the agar plate is examined. This characteristic dark colour is due to the presence of melanin pigment contained in their cell wall, which are known to be the virulent factor for these organisms4. These fungi can be isolated form soil, air, rotten wood, plants and plant roots, bird nest, staw, water, cereals in tropical and subtropical region of Africa, India, South America, Latin America and Japan2. There are more than one hundred types of dematiaceous fungi in nature. Some of the common species are *Cldosporium, Curvuleria, Madurella, Alternaria, Wangiella, Fonsecaea, Phialophora. Fonsecaea, Phialophora, Madurella, Cladosporium, Exophiala* are pathogenic to humans and animals causing rarely fatal infections in those who have normally intact host defense mechanism. Life threatening illness occur more often in immuncompromised patients. These dematiaceous fungi also contribute significantly to the morbidity and mortality of solid organ transplant recipient[6,7]. With these reports of the pathogenic potentials of dematiaceous fungi in humans and because the people of this town are largely peasant farmers, the focus of this study is to determine the prevalence of these organisms in soil samples and determine the pathogenicity of the identified isolates.

MATERIALS AND METHOD

Soil samples collected from 4 villages in Ebenebe town, Awka North L.G.A of Anambra

State, South-Eastern Nigeria were used. The 200 soil samples were randomly collected from the following towns: Umuji, 50 soil samples, Umuogbuefi 50 soil samples, Umuajani 50 soil samples and Umuoye 50 soil samples. The soil samples were collected in sterile cellophane bags using sterile spoons and processed within 24 hours of collection.

PROCESSING OF SAMPLE

One gram of each soil sample was weighed out and put into bottles containing 100mls of sterilized water. The bottles were shaked for 10 minutes and allowed to stand for 20 minutes. 1ml of the supernatant was used for the 10 folds serial dilution as previously described[8]. 1 ml each of the serial dilutions were dispensed into test tubes containing the 10^{-4}, 10^{-5} and 10^{-6} dilutions and cultured on Sabouraud dextrose agar (SDA) using the pour plate method. The plates were incubated at room temperature. The isolated colonies were then sub-cultured on agar plates.

IDENTIFICATION OF ISOLATES

The matured fungal growths were examined macroscopically and microscopically. Macroscopically established colonies were evaluated on characteristic like texture, size of colony and pigmentation if any. Also the colour of the reverse side or bottom of the plate or bottle was noted. For detailed study of the morphology and natural arrangement of the macro-conidia and conidia or arthrospores on the mycelium, slide cultures of the isolates were prepared. In the microscopic evaluation, the appearance observed was matched against those contained in colour atlases of pathogenic fungi[9,10].

PATHOGENICITY TEST

One isolate each of the dematiaceous fungi; *Fonsecaea dermatitidis, Madurella mycetomi Madurella grisea, Cladosporium werncki and Leptosphaeria senegalensis* isolated from the soil sample were used for the pathogenicity test in the study. 4 weeks old mice (body weight 15 to 20g) were housed in wooden cages and fed for two weeks. A loopful each of the test isolates were dispensed into a sterilized bottle containing 5mls of sterile water and homogenized with glass beads. Then 0.5ml of this homogenized solution was used to inoculate the mice subcutaneously using 1ml syringe as described previously[11]. The cell suspensions were also cultured on sabouraud dextrose agar (SDA) which showed growth after 4 days. The mice were observed for up to 5 weeks for the presence of lesions at the inoculation sites. The mice were sacrificed at the end of the 5th week and samples collected from the subcutaneous lesion were cultured and observed for possible recovery of the organisms.

RESULTS

Five species of dematiaceous fungi were recovered form the 200 samples of soil investigated. *Madurella grisea* was the most frequently recovered 20 (43%) followed by *Fonsecaea dermatitidis* 10 (21.7%), *Cladosporium werneckii* 10 (21.7%), *Madurella mycetomi* 5 (10.8%) and *Leptospaeria senegalensis* 1 (2.17%). Table 2 shows that most of the isolates were recovered from loamy soil (21 (50%). Table 3 shows that the samples collected from Umuji village yielded most of the dematiaceous fungi 19 (14.3%) while the samples from Umuogbuefi yielded the lowest number of organism 8 (17.3%). There were no mortality in the inoculated mice. However, *Madurella grisea and Fonsecaea dermatititidis* produced induration at the site of inoculation on the experimental mice. The organism were recovered from the subcutaneous tissue taken from the site of lesion.

Table 1: Fungal Isolates from soil and their frequencies of occurrence

Dematiaceous fungi	Frequency	% of spp isolated
Madurella grisea	20	43.4
Madurella mycetomi	5	10.8
Leptospaeria senegalensis	1	2.17
Fonsecaea dermatititidis	10	21.7
Cladosporium werneckii	10	21.7
Total	46	100%

Table 2: Distribution of dematiaceous fungi isolated according to different soil types

Isolates	Loamy soil	Clay soil	Sandy soil
Madurella grisea	10	4	6
Madurella mycetomi	5	-	-
Leptospaeria senegalensis	1	-	-
Fonsecaea dermatititidis	3	5	2
Cladosporium werneckii	6	2	2
Total	21 (50%)	11(26.26%)	10 (23.8%)

Table 3: Distribution of dematiaceous fungi isolated according to area of collection

Isolates	Umuji	Umuogbefi	Umuonye	Umuajana
M. grisea	10	4	3	3
M. mycetomi	4	-	-	1
L. senegalensis	1	-	-	-
F. dermatititidis	2	2	5	1
C. werneckii	2	2	2	4
Total	**19 (41.3%)**	**8 (17.3%)**	**10 (21.7%)**	**9 (19.5%)**

DISCUSSION

The study showed that soil serve as important natural reservoir of dematiaceous fungi. This is in conformity with the report of isolation of these fungi from soil in other countries[4,6,8,12]. The study also showed that dematiaceous fungi were more frequently isolated form loamy soil than sandy and clay soil. This is due to the more organic nature of loamy soil[13]. The only isolate of *L. senegalensis* was from loamy soil.

The five species of dematiaceous fungi isolated where more frequently isolated from Umuji village than from the other three villages. This may be due to the differences in the microbial habitat or microbial ecology of the samples. Microbial habitat is the physical location in the environment to which an organism has adapted and this depends on the climate, temperature, moisture, microbial action, pH and mineral content of the soil[4]. Although *L. senegalensis* was isolated only at one site in this study, but have been isolated more frequently from Senegal and Chad and this is why mycetoma, the infection caused by these fungi are common in the area.[15] The possible reason could be due to difference in climatic condition. This finding indicates that inhabitant of Umuji village that are mostly farmers have a high risk of contacting the infection caused by these fungi than the inhabitants of the other three villages.

The pathogenicity of the species of dematiaceous fungi isolated was confirmed in this work by the ability of *M. grisea and F. dermatitidis* to produce subcutaneous lesion in mice. Similar report has been made by Okeke et al[14] in their isolation of dematiaceous fungi *F. pedrosoi, Cladosporium carrionii, Phialophora verrucosa* from soil samples collected in Nsukka South Eastern Nigeria that produced similar lesion on mice. This shows that the microbial ecology of the South Eastern Nigeria may be similar. Gugnani also has reported

isolation of *M. mycetomi* from soil in India and which also was pathogenic to laboratory mice[8]. This support the information from literatures of the pathogenicity of dematiaceous fungi in humans[2,3,4,5,6].

These findings indicate that the study area habour dematiaceous fungi, therefore the inhabitant of the three villages have a high risk of contacting the infections caused by these fungi. There is need therefore for an epidemiological study to be carried out on the population to determine the prevalence rate of the cutaneous, subcutaneous and systemic infections caused by these pathogenic fungi.

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