

# STUDY OF THE ETIOLOGIC AGENTS OF MYCETOMA AND SPOROTRICHOSIS IN THE NORTH OF IRAN (GILAN AND MAZANDARAN PROVINCES)

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## ABSTRACT

During 2 years, from March 1987 to April 1989, 600 samples of soil and plant were collected from different cities of northern provinces of Iran. Out of the six hundred samples 58 Actinomycetes, 9 *Pseudallescheria boydii*, 10 *Sporothrix schenckii*, and 4 *Sporothrix schenckii*-like fungi were isolated as the following: *Streptomyces griseus* from 31 samples (38.27%), *Nocardia asteroides* from 8 samples (9.87%), *Actinomadura madura* from 8 samples (9.87%), *Streptomyces lavendulae* from 6 samples (7.4%), *Nocardia autotropica* from 3 samples (3.7%) *Streptomyces rimosus* from 1 sample (1.24%), *Nocardia carnea* from 1 sample (1.24%), *Pseudallescheria boydii* from 9 samples (11.11%), *Sporothrix schenckii* from 10 samples (12.34%), *Sporothrix schenckii*-like fungi including *Ceratocystis* sp. from 2 samples (2.48%), *Doratomyces purpureofuscus* from 1 sample (1.24%), and *Scopulariopsis candida* from 1 sample (1.24%).

MJIRI, Vol.5, No. 3 & 4, 139-143, 1991

## INTRODUCTION

The genera of *Nocardia* and *Streptomyces* are well known causes of human and animal disease. Members of both genera have been responsible for localized infection in the form of mycetoma, while some *Nocardia* spp. predominantly *Nocardia asteroides* have been incriminated as the causes of more severe systemic mycosis with primary foci in the lung.<sup>1</sup> Soil is well known to be the natural habitat of these organisms and constitutes the main reservoir of infection.<sup>1</sup> Mycetoma has been known for years in Iran. The first case was identified in 1962 and 34 cases have been reported until 1989.<sup>2</sup>

*Sporothrix schenckii* is an organism commonly found on decaying vegetation, and it has been repeatedly isolated from the soil.<sup>3</sup> Infection is usually acquired by traumatic implantation of spores into the skin or subcutaneous tissue.<sup>4</sup> The first case of sporot-

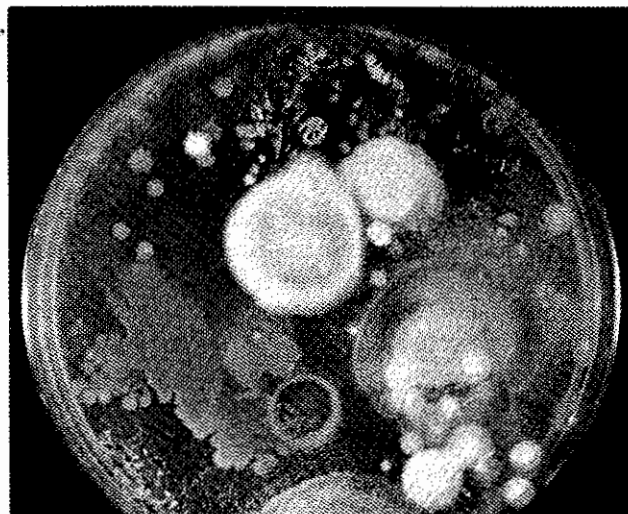


Fig. 1. Mycelial colonies of *Sporothrix schenckii* and strains of Actinomycetes, isolated from the soil and plant samples by agar plating method.

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richosis was identified in Iran in 1983 and seven cases have been diagnosed up to the present time.<sup>5</sup> Although mycetoma and sporotrichosis have been known in this country, the etiologic agents of these diseases have not been isolated from the nature in Iran.

The main purpose of this research is to study the frequency of the etiologic agents of mycetoma and sporotrichosis in the nature of Gilan and Mazandaran provinces.

### MATERIALS AND METHODS

During 2 years 600 samples of soil and plant were collected from different cities of Gilan and Mazandaran provinces (Babol, Amol, Ghaemshahr, Sari, Behshahr, Gorgan, Fereidonkenar, Neka, Mahmoodabad, Chalus, Noshahr, Tonkabon, Marzanabad, Keldasht, Amlash, Nashtarood, Chaboksar, Klachi, and Roodsar). These samples were collected from these areas either at random or from areas where the human mycetoma and sporotrichosis were reported.

Soils were collected from just below the surface and stored in sterile plastic bags. Also plant samples such as tree barks, sphagnum moss, dry grasses, rose, and raspberry bushes were collected from these areas. After collection of the specimens, they have been brought to the laboratory and stored in refrigerator. Soil suspensions were prepared by adding five grams of soil to 30 ml of isotonic saline solution and glass beads. After five minutes of vigorous shaking, the suspension was left for 10 minutes. The heavier particles settled on the bottom while plant debris floated on the surface. There remained a suspension of small particles of which 8 ml was transferred to a test tube containing 0.5 gram cycloheximid. Pathogenic fungi were isolated from this suspension by direct culture. Culture was made on duplicate Sabouraud's glucose agar (S), and Sabouraud's glucose agar containing cycloheximid (0.5gr/l) (Sc). Two or three drops of the suspension were spread over the plate and then incubated at 37°C and 30°C. The plant specimens were inoculated directly on the S and Sc media. Suspicious colonies were transplanted into another tube with the same medium. The parafin-bait technique has been successful for the recovery of *Nocardia* spp. from soil by several workers. We found that it had limitations since many saprophytic moulds grew as contaminations, so we compared the parafin medium with S and Sc medium for the isolation of actinomycetes from the soil and plants.

We found that S and Sc media are better than parafin medium so we used only S and Sc medium for isolating all pathogenic fungi.

I. Methods performed for the identification of aerobic actinomycetes included: slide culture and stain with



Fig.2. Strains of actinomycetes, isolated from soil and plants samples, north of Iran.

Kinyoun methods, decomposition of casein, tyrosine, xanthine, hypoxanthine, and adenine; acid production from arabinose, cellobiose, erythritol, glycerol, lactose, maltose, mannitol, melibiose, sorbitol, and xylose; hydrolysis of esculin, gelatin, and starch.

II. Methods performed for the identification of eumycetoma agents included: slide culture and animal inoculation for virulence studies.

III. Methods performed for the identification of *Sporothrix schenckii* and *Sporothrix schenckii*-like fungi included: Slide culture, test for pigmented colonies

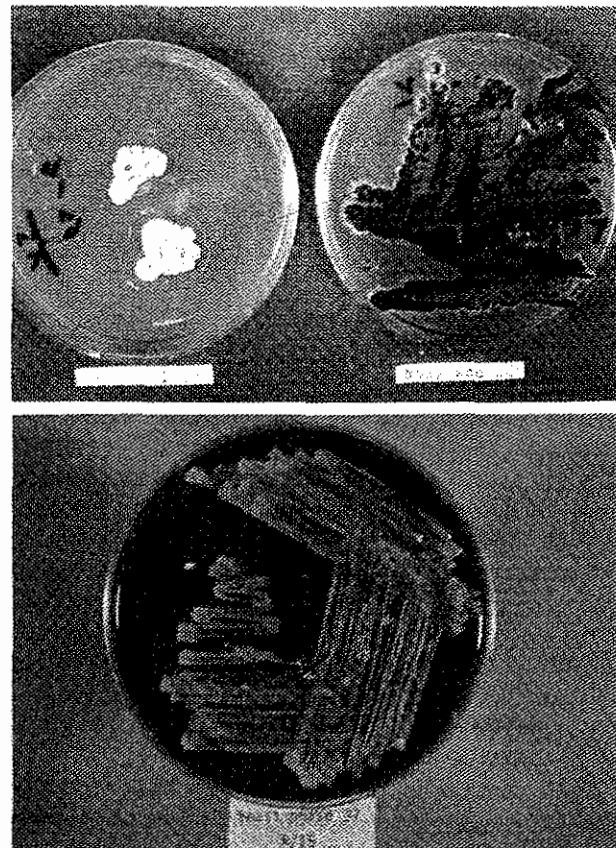


Fig.3,4. Mycelial and yeast phases of *Sporothrix schenckii*, isolated from soil and plant samples, north of Iran.

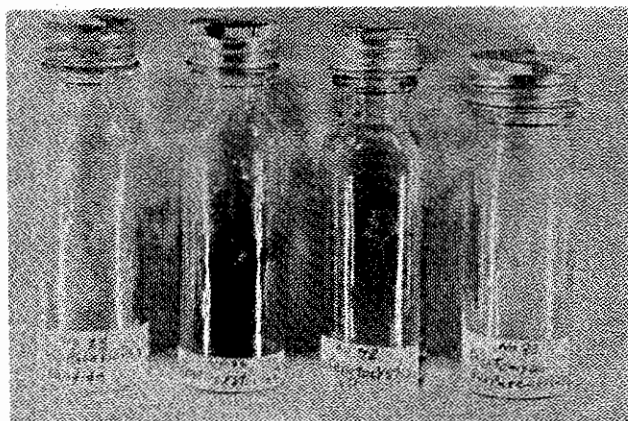


Fig.5. Strains of *Sporothrix schenckii*-like fungi, isolated from soil and plant samples, north of Iran.



Fig.6. Black pigmented colonies of *S. schenckii* on corn-meal agar medium.



Fig.7. Periorchitis caused by *Sporothrix schenckii* 12 days after inoculation of mycelial colony.

produced in corn-meal agar animal inoculation for virulence studies, subculture the colonies into BHI and blood for observation of the ability of the fungi to grow in a yeast cell phase.

#### Pathogenicity tests

A dense suspension of 0.5 ml, obtained by finely triturating small amounts of *Pseudallesheria boydii* colonies with sterile saline, was injected intraperitoneally into eight mice, which were kept for a period of up to one month. After this period the animals were sacrificed and fragments of lung, liver, spleen and kidneys were cultured on the S medium. Also tissue sections of these organs were performed.

10 male hamsters were inoculated with 0.2 ml of *Sporothrix schenckii* suspension in their testicles. Whenever orchitis was found to have developed in the inoculated hamsters, the animals were killed (12 days later). The exudate was examined and tissue sections were performed. Culture of inoculated testis were made on the S, SC, and BHI (brain, heart infusion agar) medium.

## RESULTS

Out of six hundred soil and plant samples, 58 actinomycetes, one *Pseudallesheria boydii*, 10 *Sporothrix schenckii* (Fig.1), four *Sporothrix schenckii*-like fungi were isolated as the following:

- Streptomyces griseus* from 31 samples (38.27%)
- Nocardia asteroides* from eight samples (9.87%)
- Actinomadura madura* from eight samples (9.87%)
- Streptomyces lavendulae* from six samples (7.4%)
- Nocardia autotropica* from three samples (3.7%)
- Streptomyces rimosus* from one sample (1.24%)
- Nocardia carnea* from one sample (1.24%) (Fig.2)
- Pseudallesheria boydii* from nine samples (11.11%)

*Sporothrix schenckii* from 10 samples (12.34%) (Fig. 3,4)

*Sporothrix schenckii*-like fungi including ceratocystis sp. from two samples (2.48%)

*Doratomyces purpureofuscus* from one sample (1.24%)

*Scopulariopsis candida* from one sample (1.24%) (Fig.5)

The intraperitoneal injection of mice with spore suspensions of *Pseudallesheria boydii* isolated in this study, failed to produce any pathologic changes. Injected strains were not obtained from the spleen and liver cultures.

All strains of *Sporothrix schenckii*, isolated from soil and plants, hardly grew at 37°C. On corn-meal agar medium they produced pigmented conidia (Fig.6) and

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were pathogenic for the hamster. After inoculation of the mycelial phase into the testis of hamster, periorchitis developed 12 days later (Fig.7). In the section of this organ granulomatous reaction and asteroid bodies were seen with giemsa staining (Fig.8).

Giemsa stain of pus from testis demonstrated rounded and elongated budding yeast cells (Fig.9).

On S, Sc, and BHI medium these strains were isolated from the testis of injected hamsters (Fig.10). Strains of *Sporothrix schenckii*-like fungi isolated in this research, were not able to produce black pigmented colonies in corn-meal agar medium and did not produce orchitis in the injected hamster.

### DISCUSSION

The results of this survey provide information for the first time on the prevalence of the etiologic agents of mycetoma and sporotrichosis in the soil and plants of the northern provinces (Gilan and Mazandaran) of Iran. Most of the mycetoma cases and some cases of sporotrichosis have been reported from the rainy and humid states in the north of Iran. Environmental condition such as the temperature and humidity of the area must be suitable for the growth of these pathogenic organisms in the soil. In this country actinomycetoma is significantly more prevalent.<sup>7-8</sup> In a study, out of 13 positive cases only one case of eumycotic mycetoma was found.<sup>2,9</sup> In this research 58 strains of actinomycetes and only one strain of *Pseudallescheria boydii* were isolated from soil and plant samples. The most common species, *Streptomyces griseus*, was found in 38.27% of the soil samples investigated. *Nocardia asteroides* is most frequently the etiologic agent for nocardiosis but *Streptomyces griseus* has been associated with the diseases. *Nocardia asteroides*, *Actinomyces madura* and *Streptomyces griseus* are often isolated from the upper respiratory tract without clinical evidence that they are contributing to a disease process.<sup>10</sup> *Nocardia asteroides* and *Actinomyces madura* have been reported as the most common causes of mycetoma in Iran.<sup>2,7</sup>

We isolated eight strains of *N. asteroides* and eight strains of *A. madura* from the soil and plant samples. All the *S. schenckii*, isolated in this research, were pathogenic for the hamster, producing periorchitis. Some non-pathogenic strains of graphium, ceratocystis and others, when grown on Sabouraud's glucose agar exhibited some similarities to non-pigmented or pale strains of *S. schenckii*. These strains do not produce pigmented conidia when grown on corn-meal agar. They produce non-pigmented oval or pear-shaped conidia. After six to seven days *S. schenckii* produced a blackish flat dull growth. Graphium and other

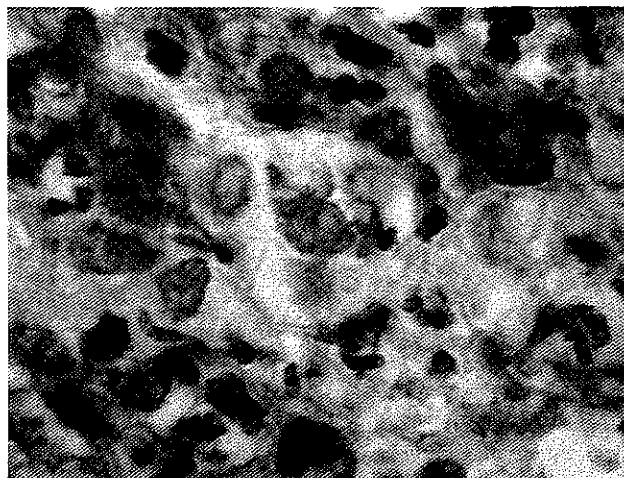


Fig.8. Asteroid body in the testicular tissue of a hamster (H and E, × 1000).

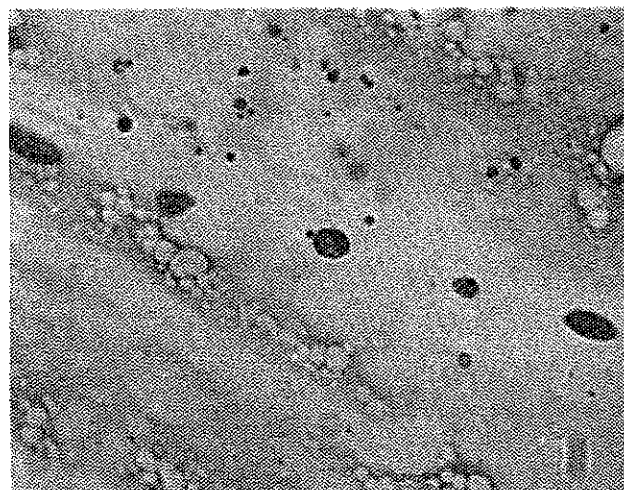


Fig.9. Rounded budding yeast cells of *S. schenckii* in the smear of hamster's testis pus (Giemsa stain × 1000).

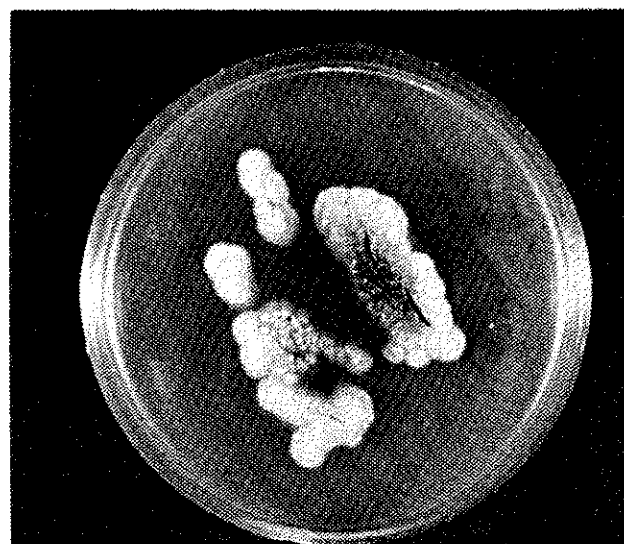


Fig.10. Mycelial phase of *Sporothrix schenckii*, isolated from the testicular tissue of hamster on S medium.

sporotrichum-like species produce a filamentous whitish growth.<sup>11,12</sup> In the study we isolated four strains of *S. schenckii*-like fungi including *ceratocystis* sp., two strains, *Doratomyces purpureofuscus* one strain, and *Scopulariopsis candida*, one strain. None of which were able to produce lesion in the testis of hamsters after inoculation, and they were not isolated from this organ on S, Sc, and BHI medium.

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