



Rare Case series of Eumycetoma (Black and white granule mycetoma) among Post Covid-19 and immunosuppressed individuals

¹Dr Mayuri Mahajan, ²Dr Prasanna S, ³Dr Nikunj Kumar Das, ⁴Dr Manjiri Khade, ⁵Dr Dipak Malokar

¹Consultant and Head, ^{2,3}Associate Professor, ⁴Assistant Professor, ⁵Director

¹Dr Hedgewar Hospital and Research Centre, Akola

²Microbiology, Shri Sathya Sai Medical College and Research Institute, Sri Balaji Vidyapeeth (Deemed to be University), Chengalpattu, Tamil Nadu-603108, ³Microbiology, D.Y.Patil Medical College, Pune Pathology, ⁴GMC, Akola, ⁵Malokar Centre for Orthopaedic hand and microsurgery, Akola

*Corresponding Author:

Dr Prasanna S

Microbiology, Shri Sathya Sai Medical College and Research Institute, Sri Balaji Vidyapeeth (Deemed to be University), Chengalpattu, Tamil Nadu-603108

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Abstract

Mycetoma is frequently seen in clinical practice worldwide, mainly in the rural areas and is usually occupation related. Mycetoma is chronic granulomatous infection of skin and subcutaneous tissues with involvement of underlying fasciae and bones in majority of cases. Lower extremities are the most common site involved by mycetoma. Commonly, it is caused by two groups of organisms – fungus and bacteria. The etiological agent varies belonging to several genera, species and based on the granules produced into white and black granule

eumycetoma. The objective of this case series is to present Eumycetoma (Black and white granule mycetoma) among immunosuppressed individuals by *Aspergillus nidulans*, *Aspergillus niger*, *Alternaria alternata*, *Curvularia lunata*, and *Mucor species*

Keywords: Eumycetoma, *Aspergillus nidulans*, *Aspergillus niger*, *Alternaria alternata*, *Curvularia lunata*, and *Mucor species*

INTRODUCTION

Mycetoma is a frequent fungal infection encountered in dermatological practice. The clinical picture is slowly progressive, causing chronic granulomatous infection of skin and subcutaneous tissues with involvement of underlying fasciae and bones usually affecting the extremities.¹ This disease is defined by the triad of tumefaction of affected tissue, formation of multiple draining sinuses and presence of oozing granules.^{1,2,3} In most cases it is caused by two groups of organisms - fungus (eumycetoma) and bacteria (actinomycetoma and botryomycosis). The eumycetoma is classified into black granule eumycetoma and white granule eumycetoma. The white granule mycetoma is caused by more than 10 species of fungi such as *Acremonium species*,

Pseudoallescheria boydii, *Aspergillus nidulans*, *Aspergillus flavus*, *Fusarium species* and *Scedosporium apiospermum*. The black granule mycetoma is caused by *Madurella mycetomatis*, *Madurella grisea*, *Exophiala jeanselmei*, *Curvularia geniculata*, *Alternaria alternata*.^{1,2,3} Mycetoma is worldwide in distribution, mostly seen in rural areas and is mainly occupation related. The prevalence of mycetoma varies widely being more frequent in hot, humid, tropical and sub-tropical areas. Mycetoma is endemic in Africa, Sudan, Senegal, southern and northern parts of India. As there is a huge difference between treatment of mycetoma due to fungal and bacterial cause, identification of the etiological agent plays a major role in the management.^{2,3,4,5,6} Upto our

knowledge this will be first case series causing white granule and black granule eumycetoma over different sites as compared to routine (leg and foot) like back of shoulder, maxilla and mandible by various rare group of fungi like *Aspergillus nidulans*, *Aspergillus niger*, *Alternaria alternata* and *Curvularia lunata*, among post covid-19 and immunosuppressed individuals.

CASE SERIES DESCRIPTION:

Case 1:

A 55-year-old male diabetic patient, presented with multiple lumps and nodules over right lower limb for three months. The patient developed progressive diffuse painful swelling of right leg till knee. Discharging fluid was clear and sometimes white coloured granules present (Fig. 1). Associated symptoms were fever with chills and patient was admitted to a hospital. The past history was post COVID 19 patient moderate category in the first wave and treated with steroids. The white granules and discharge fluid were sent for bacterial and fungal cultures. The patient was managed with surgical debridement and antibiotics.

The microscopic examination of granules after treatment with 40% KOH revealed the presence of septate hyaline hyphae. The white granules were cultured in Sabouraud dextrose agar plates with and without chloramphenicol and were incubated at 25-30°C. After seven days of incubation, the colonies were powdery with dark green colour surrounded by white brim (Fig. 2). Microscopy by Lactophenol cotton blue (LPCB) mount showed non-radiating biserial phialides on upper part and several hulle cells (Fig. 3).

The slide culture techniques were set up for detailed morphological study. LPCB mount of slide culture showed hemispherical vesicles of size 7-8 µm in diameter, non-radiating biserial, spherical, rugose, sub-hyaline conidia. The fungus was identified as *Aspergillus nidulans* with the help of KOH mount, cultural characteristics, LPCB mount and wet mount features, with the presence of hulle cells clinching the diagnosis. The same fungus was isolated again from repeat sample of skin scrapings. The patient was put on itraconazole, showed improvement after four weeks and to be followed up further.

Case 2:

A 67-year-old male patient, presented with multiple sinuses and back granules over right lower limb (lateral and dorsum of foot) for one month. The patient developed progressive diffuse painful swelling of right foot. Discharging fluid was clear and sometimes black- and grey-colored granules present (Fig. 1). The patient was admitted to a hospital and also had history of fever. The patient having history of treatment with steroids and anti-rheumatoid arthritis drugs. The black granules and discharge fluid were sent for bacterial and fungal cultures. The patient was managed with surgical debridement and antibiotics.

The microscopic examination of granules after treatment with 40% KOH revealed the presence of septate hyaline hyphae. The Gram stain showed the absence of bacteria and no acid-fast bacilli by Ziehl Neelsen stain. The material was cultured in Sabouraud dextrose agar (SDA) plates with and without cycloheximide. SDA without cycloheximide grew Suede like Olive-black or greyish appearance and fast growing after about 7 days (Fig. 4). Lactophenol cotton blue (LCB) mount showed hyphae with branched acropetal chains of multicellular conidia (dictyoconidia) are in single or branched and short or elongate conidiophores. Conidia are smooth walled, clavate, pyriform, sometimes ovoid or ellipsoidal, often with a short conical or cylindrical beak (Fig. 5). The fungus was identified as *Alternaria alternata* with the help of cultural characteristics and the typical microscopic features of hyphae on LCB mounts. The same fungus was isolated again from repeat sample. The patient was managed with below knee amputation, antifungals like I.V amphotericin B, antibiotics and discharged with oral Itraconazole.

Case 3:

A 22-year-old male patient, presented with multiple sinuses and back granules over right side back of the shoulder and mandible and cheek for 20 days. Discharging fluid was clear and sometimes black coloured granules present. The patient was admitted to a hospital and also had history of fever. The patient is COVID 19 positive, admitted and treated as moderate category with steroids. The black granules and discharge fluid were sent for bacterial and fungal cultures. The patient was managed with wound dressing and antibiotics.

Table 1: The black and white grain Eumycetomas and causative organisms ^{1,2,3,12}

Eumycetoma	Fungi responsible
Black grain	<i>Madurella mycetomatis</i> , <i>M. grisea</i> , <i>Leptosphaeria senegalensis</i> , <i>L. tompkinsii</i> , <i>Exophiala jeanselmei</i> , <i>Pyrenochaeta romeroi</i> , <i>Curvularia lunata</i> , <i>Phialophora verrucosa</i> , <i>Phytophthora parasitica</i> , <i>Cladophialophora bantiana</i> , <i>Aspergillus terreus</i> , <i>A. niger</i>
White grain	<i>Pseudallescheria boydii</i> , <i>Aspergillus nidulans</i> , <i>A. flavus</i> , <i>A. fumigatus</i> , <i>Fusarium Species</i> , <i>Acremonium Species</i> , <i>Neotestudina rosatii</i> , <i>Scedosporium apiospermum</i>



Figure 1- White granule (first two images) and black granule (last two images) eumycetoma with discharging sinuses

The microscopic examination of granules after treatment with 40% KOH revealed the presence of septate hyaline hyphae. The Gram stain showed the absence of bacteria and no acid-fast bacilli by Ziehl Neelsen stain. The material was cultured in Sabouraud dextrose agar (SDA) plates and slant tubes with and without cycloheximide. SDA without cycloheximide grew smooth, white colonies initially and matures to velvety, jet-black center with characteristic white apron or brim and no reverse pigment after about 7 days (Fig. 6). Lactophenol cotton blue (LCB) mount showed hyphae with black colour, erect conidiophores terminated with radiating phialides covering entire vesicles (Fig. 7). The fungus was identified as *Aspergillus niger* with the help of cultural characteristics and the typical microscopic features of hyphae with black colour, erect conidiophores terminated with radiating phialides covering entire

vesicles on LCB mounts. The same fungus was isolated again from repeat sample of skin scrapings. The patient was managed with wound dressing, antibiotics and oral itraconazole for four weeks

Case 4:

A 51-year-old male patient, presented with multiple sinuses and back granules over right lower limb (lateral and dorsum of foot) for 2 months. The patient developed progressive diffuse painful swelling of right foot and non-healing ulcerative lesions. Discharging fluid was clear and sometimes black granules present. The patient was admitted to a hospital and also had history of fever. The past history was post COVID 19 patient moderate category in the first wave and treated with steroids. The black granules and discharge fluid were sent for bacterial and fungal cultures. The patient was managed with wound dressing and antibiotics.



Figure 2- Growth of *Aspergillus nidulans* on Sabouraud dextrose agar showing typical dark green colour centre white brim, powdery and wrinkled colonies

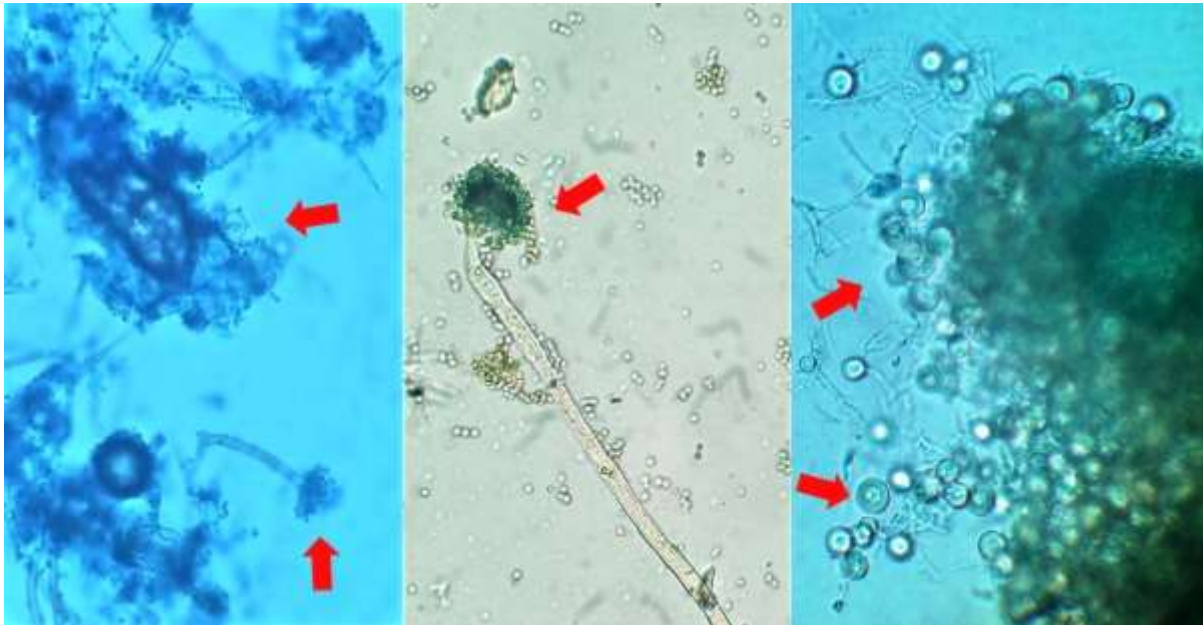


Figure 3- Lactophenol cotton blue mount of *Aspergillus nidulans* showing biseriate phialides on upper part non radiating and also hulle cells (red pointed arrows)



Figure 4- Growth of *Alternaria alternata* on Sabouraud dextrose agar showing Suede like Olive-black or greyish appearance

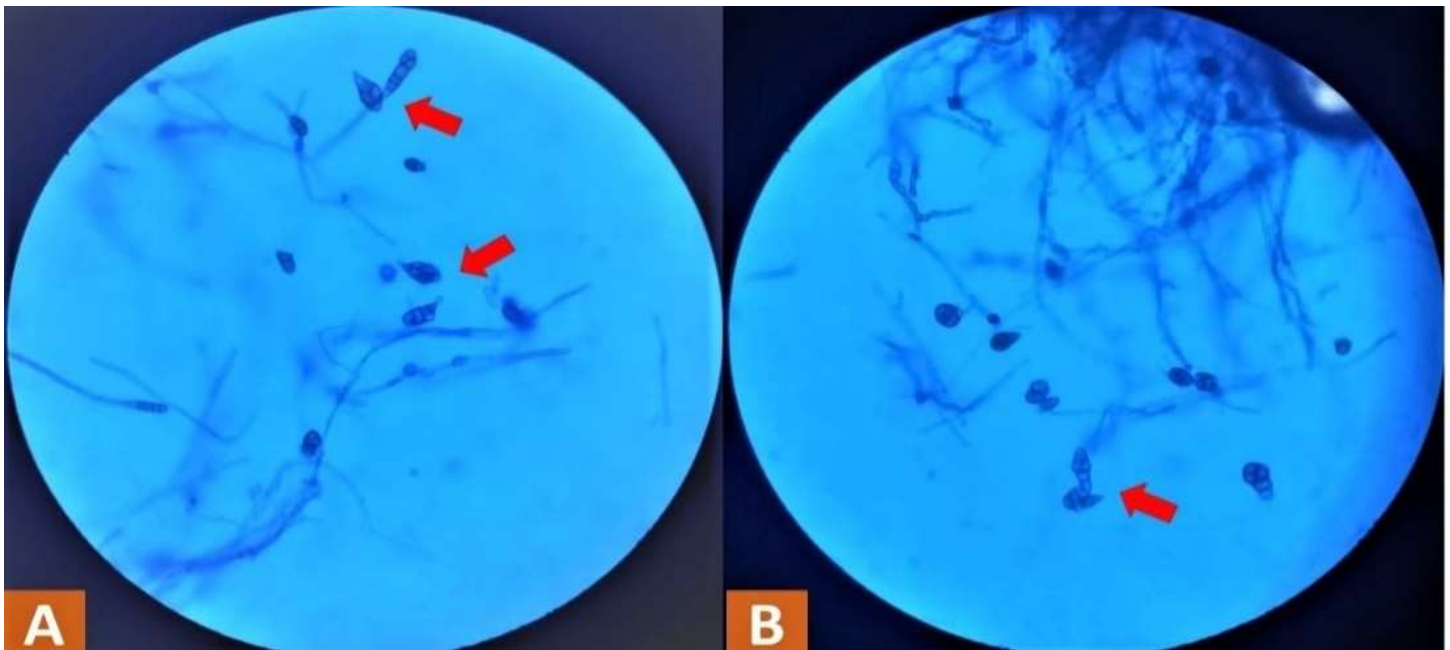


Figure 5- Lactophenol cotton blue mount of *Alternaria alternata* showing hyphae with branched acropetal chains of multicellular conidia are in single or branched and short or elongate conidiophores. Conidia are smooth walled, clavate, pyriform, sometimes ovoid or ellipsoidal, often with a short conical or cylindrical beak (red pointed arrows)



Figure 6- Growth of *Aspergillus niger* on Sabouraud dextrose agar showing white colonies initially and matures to velvety, jet black center with characteristic white apron or brim and no reverse pigment

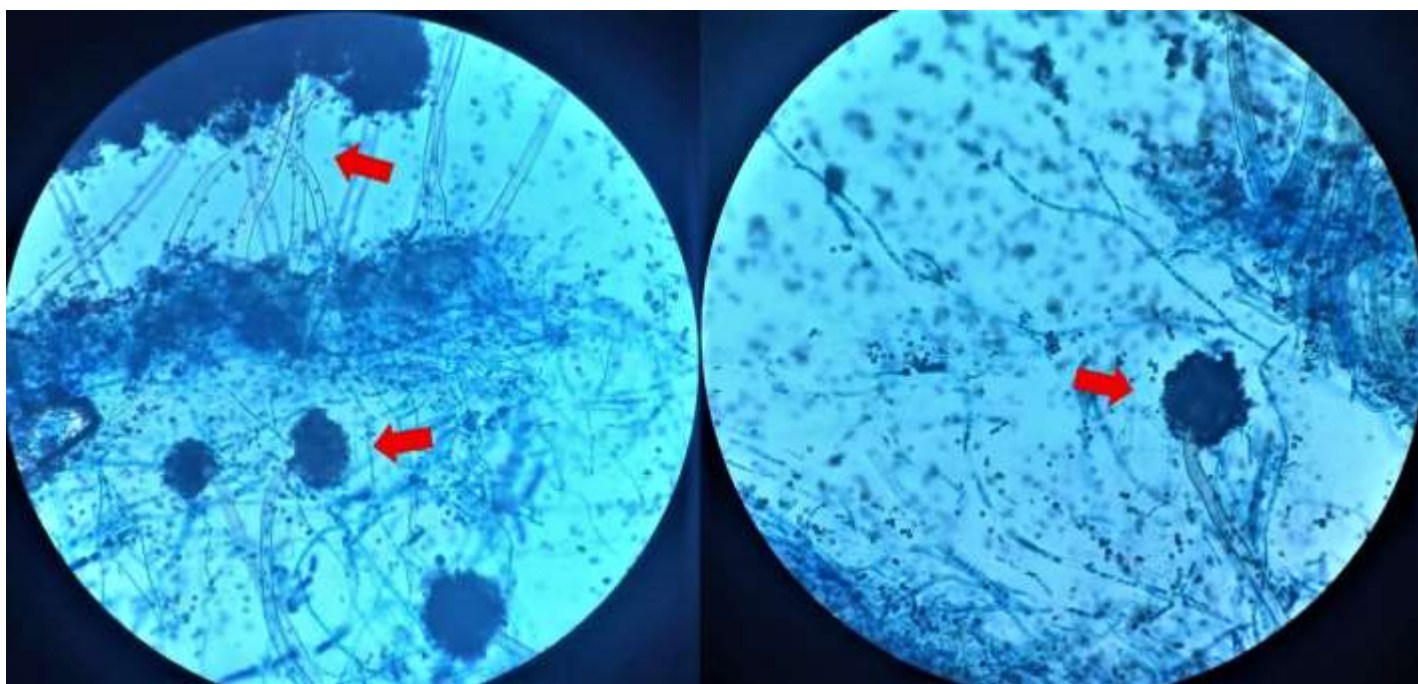


Figure 7- Lactophenol cotton blue mount showing hyphae with black colour, erect conidiophores terminated with radiating phialides covering entire vesicles (red pointed arrows)- *Aspergillus niger*



Figure 8- Growth of *Curvularia lunata* on Sabouraud dextrose agar initially grown as downy white to green and later fast growing after about 7 days changes its colour to olive brown-black

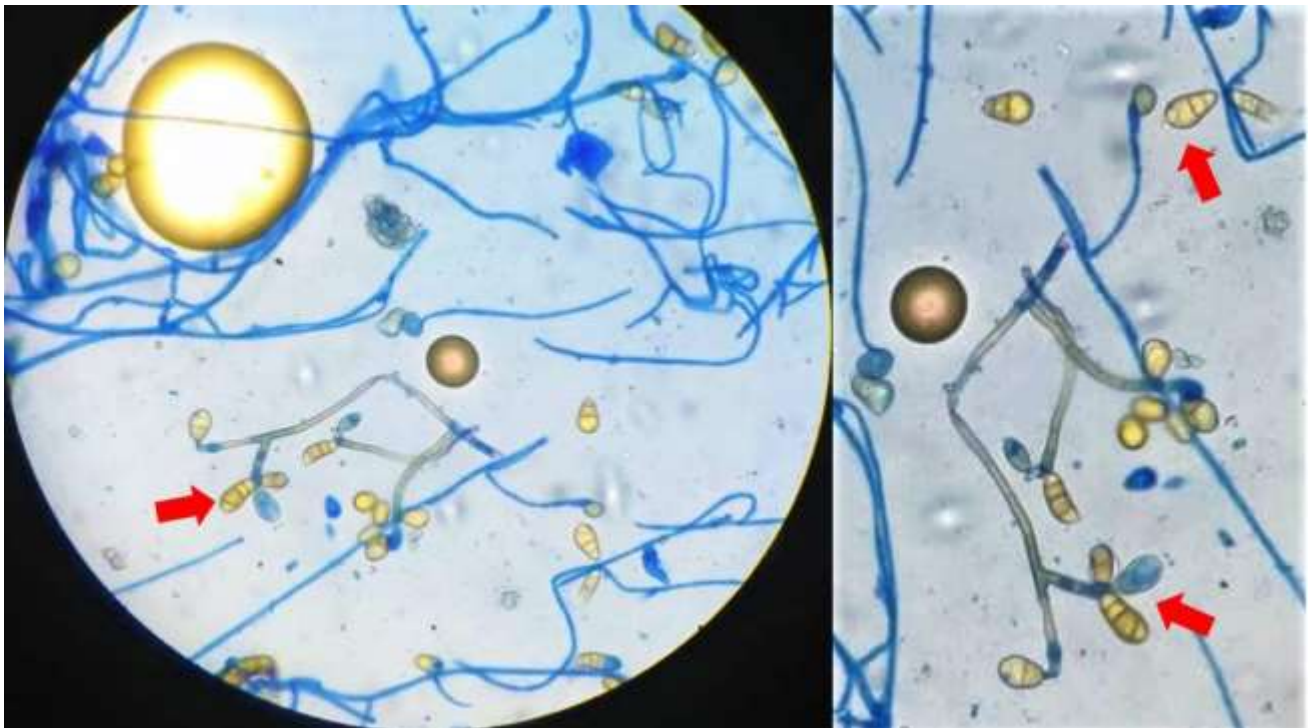


Figure 9- Lactophenol cotton blue mount of *Curvularia lunata* showing Conidiophores erect, branched or unbranched, septate. Conidia are smooth-walled, olive-brown, ovoid to broadly clavate, curved at the sub-terminal cells (swollen and distinctly larger than the remaining cells)- red pointed arrows

The microscopic examination of granules after treatment with 40% KOH revealed the presence of septate hyaline hyphae. The Gram stain showed the absence of bacteria and no acid-fast bacilli by Ziehl Neelsen stain. The material was cultured in Sabouraud dextrose agar (SDA) plates with and without cycloheximide. SDA without cycloheximide initially grown as downy white to green and later fast growing after about 7 days changes its colour to olive brown-black (Fig. 8). Lactophenol cotton blue (LCB) mount showed Conidiophores erect, branched or unbranched, septate. Conidia are smooth-walled, olive-brown, ovoid to broadly clavate, curved at the sub-terminal cells (swollen and distinctly larger than the remaining cells) (Fig. 9). The fungus was identified as *Curvularia lunata* with the help of cultural characteristics and the typical microscopic features on LCB mounts. The same fungus was isolated again from repeat sample. The patient was managed with wound dressing, antibiotics and oral itraconazole for four weeks.

DISCUSSION:

The mycetoma foot is prevalent in almost all parts of the world, mainly between tropics of Cancer and Capricorn. Mycetoma is said to be more prevalent in places where rainfall is more and with temperature of 15 to 25°C. In India, mycetoma is distributed in widely different geographical areas like Tamil Nadu (Madurai), the moist southern part and Rajasthan.¹ But, Maharashtra is in the western part of India where mycetoma is not endemic. The various risk factors are patients with granulocytopenia, high dose corticosteroid treatment and immunocompromised status following bone marrow transplantation and chronic granulomatous disease.^{1,2,3}

Depending upon the aetiology, the eumycetoma is classified into black grain eumycetoma and white grain eumycetoma. The most common causes for black grain eumycetoma are *Madurella mycetomatis*, *Madurella grisea*, *Exophiala jeanselmei*, and *Curvularia geniculata* and *Curvularia lunata*.^{1,2,3} The white grain eumycetoma itself is very rare as compared to black grain eumycetoma and actinomycetoma.³ The white grain eumycetoma are caused by various organisms like *Pseudallescheria boydii*, *Aspergillus nidulans*, *A. flavus*, *A. fumigatus*, *Fusarium Species*, *Acremonium Species*, *Neotestudina*

rosatii, *Scedosporium apiospermum*. Different causes of eumycetoma are shown in Table 1.^{1,2,3,12}

Aspergillus nidulans is a saprophytic mould which is found in decaying organic matter. Mycetoma foot caused by this species has been known since 19th century, when Pinoy from Tunisia isolated it from cases of mycetoma. Nicolle and Blanc (1920), also confirmed the role of *A. nidulans* as etiological agent. Mycetoma due to *A. nidulans* has been reported from various parts of the world by Puestow in 1928, Baylet *et al* in 1968 from Senegal and Mahgoub from Sudan in 1971.^{4,6,7,9,10} In India, mycetoma due to *A. nidulans* is rare with only a few case reports.

Around 180 different species of *Aspergillus* are present, they are filamentous moulds, opportunistic fungi. These are ubiquitously distributed in water, soil, air, plants, dust, fields, deserts etc. Infection in human is caused by many species of *Aspergillus*. Many physical and chemical properties are shared among 15 related black spores forming species of *Aspergillus niger* and they belong to the Section *nigri*, also known as black moulds.¹³

Alternaria belongs to weak and pigment or melanin producing pathogen known as dematiaceous fungus. The predisposing factors for *Alternaria alternata* soft tissue infections are hematological malignancies, systemic lupus erythematosus, rheumatoid arthritis, organ transplantation, HIV, diabetes mellitus and other immunosuppression status and prolonged steroid treatment cases.¹⁴ In literature till now only two or three case reports of *Alternaria* species causing mycetoma have been reported. So, third or fourth was ours with this case series of eumycetoma one among the causative agent as *Alternaria alternata*. In patient with lymphocytic leukemia, a progressive subcutaneous lesion caused by *Alternaria alternata* which was not subsiding with voriconazole and micafungin was reported by Kpodzo DS *et al*.^{14,15} But later eradication achieved after surgical resection and posaconazole treatment. Another case of *Alternaria* species producing erythematous scaly lesion over foot and which recovered with oral itraconazole therapy for 6 weeks period was reported by Gurcan *et al* in immunocompetent individual.^{14,16}

Dematiaceous fungi like *Curvularia* species are ubiquitously seen in soil and vegetation. Many species of *Curvularia* upto 31 species were isolated in that most frequently reported as pathogens were

Curvularia lunata and Curvularia geniculata. Initial era of mycological studies Curvularia was considered to be plants pathogens, but recently they dominating as human infection causing agents. The most commonly encountered infections are ocular, respiratory, cerebral infections, and Mycetoma as per their frequency.¹⁷

CONCLUSION:

The diagnosis of complicating diseases such as eumycetoma and its etiological fungi at earlier is the key to initiation of prompt management. The usual eumycetoma will affect the extremities like foot, legs and hands, here we reported case series that also affected unusual sites like back of shoulder, maxilla and mandible. The management of white and black granule eumycetoma in post covid-19 and immunocompromised individuals with various rare group of fungi like *Aspergillus nidulans*, *Aspergillus niger*, *Alternaria alternata* and *Curvularia lunata* will be quite interesting and challenging, because some of them are residents of soil and plant pathogens.

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