Indoor aeromycoflora of Baroda museum and deterioration of Egyptian mummy

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There are a number of abiotic and biotic agencies like pollution, light, humidity, temperature, actinomycetes, algae, fungi, bacteria, insects, etc. that have a deteriorating effect on museum materials. Of these agencies, biological agents such as microorganisms and insects may cause devastating damages.

A study of indoor aeromycoflora of the Baroda Museum and Picture Gallery, Vadodara was undertaken during 1999–2000. Samples were also taken from dust present in the coffin box and body parts of an Egyptian mummy (dated about 230 BC). Twenty-two viable species of fungi belonging to 17 genera were isolated in May 2000 from indoor air of Egypto-Babylonian Gallery by gravity fall method. The isolations from air and dust of the mummy chamber resulted in 6 different fungi. The mycoflora was dominated by different Aspergilli. Isolations from the first toe of the right leg of an 1.54 m long Egyptian mummy revealed the presence of *Emericella nivea* Wiley & Simmons. This fungus produced white powdery patches on the toes of the mummy. In culture, both anamorphic and teleomorphic stages were observed. The paper deals with occurrence of fungal deteriogens in indoor air of the Baroda Museum and Picture Gallery and biodeterioration of one of the seven unique museum objects present in our country.

BIODETERIOGENS like fungi, bacteria and actinomycetes pose severe threat to museum objects. Microbes get entry into indoor atmosphere through wind currents and settle on various objects by impaction[1]. These are disseminated by the action of wind and cause severe losses to valuable historical and cultural property. In the new millennium, when we are concerned with conservation of these valuable objects constituting non-renewable cultural heritage, detailed studies are required to know the behaviour of these biodeteriogens and to find out methods to control them. It is interesting to note that any biodeteriorating object may act as a small or large bio-industry, emitting neither polluted water nor noxious gases, but a large number of bio-particles with a potential to start another such unit in a short span. These serve as a secondary source of biodeteriogens.

The Baroda Museum and Picture Gallery is famous for its collection of European oil paintings, all over Asia. The buildings of the museum are situated in a pastoral environment of Sayaji Park. The museum was built by Maharaja Sayajirao Gaekwad III in Indo-Saracenic style of architecture in 1894.

Study on indoor aeromycoflora has attracted the attention of numerous aerobiologists[1–7]. Scientists have emphasized the importance of monitoring such biopollutants in different indoor environments. Tilak and Kulkarni[4] studied the indoor aeromycoflora of caves in Aurangabad. The paintings of Ajanta and Ellora caves have deteriorated due to fungal growth[5,8–11]. Subbaraman[12] reported growth of fungi on paintings, when relative humidity exceeded 65%. Aerospora of these caves has been studied[9,10]. Mycological analysis of indoor air in Bharat Kala Bhawan, Varanasi[13] and libraries in Lucknow[14] have confirmed the role of biodeteriogens. Problems of biodeterioration in Baroda Museum and Picture Gallery have been recorded earlier[6,15–17]. Sarbhoy[18], describing measures employed to combat biodeterioration has mentioned, 'Although the human dead body can hardly be described as a resistant material, some of the earliest examples of combating microbial attack of perishable materials are seen in mummification'. Considering the need to monitor such biopollutants, an effort has been made during 1999–2000 in the atmosphere of the Baroda Museum and Picture Gallery. This communication reports the findings of these studies on indoor air and occurrence of a fungus on a deteriorating mummy kept in the Egypto-Babylonian Gallery.

Analysis of indoor air in the Baroda Museum was done on 22 September 1999 and 5 May 2000, using the culture plate exposure method[19]. The results are recorded in Table 1 and Figure 1. Five petri dishes (100 mm each) were exposed for 10 min in the Egypto-Babylonian Gallery. These were incubated at 25 + 1°C for 7 days. As soon as the fungal growth appeared, sub-culturing was done. Pure cultures were maintained on PDA slants. Fungi from dust were isolated by dilution plate method[20]. Species of *Aspergillus* and *Penicillium* were identified by studying their morphological characters in Czapek-Dox's medium. Scrappings from white growth on toes were transferred aseptically in culture tubes. Cellophane tape method was also tried to isolate the fungal biodeteriogens (Figure 2 *a*). The minimum and maximum relative humidity on 16 April 1999 was 18% and 63%, respectively and the room temperature at the time of sampling was 32°C. Occurrence of fungi on 16 April 1999 in the air and dust of the mummy chamber is recorded in Table 2.

The Egyptian society believed not only in existence of god and living beings, but also in the dead. Their concept was that the soul *Ba* could enter the body again. The body of the dead should not deteriorate and it should be attractive enough to lure back the soul. From the Greco-Roman period, bitumen was used in the process and it was from Persian word *Mumiyah* for bitumen that the term

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Table 1. Indoor aeromycoflora of Egypto-Babylonian Gallery in Baroda Museum and Picture Gallery in 1999–2000

Fungi isolated	September 1999		May 2000	
	Colonies*	Percentage occurrence	Colonies*	Percentage occurrence
Zygomycotina				
Absidia repens	–	–	5	3.4
Mucor hiemalis	8	4.8	5	3.4
Rhizopus stolonifer	10	6.1	5	3.4
Syncephalastrum racemosum	–	–	2	1.4
Ascomycotina				
Aspergillus flavus	5	3	10	6.8
A. japonicus	7	4.2	5	3.4
A. niger	17	10.3	20	12.2
A. parasiticus	–	–	7	4.8
A. ustus	5	3	–	–
A. wentii	7	4.2	–	–
Cheatomium atrobrunneum	6	3.6	–	–
C. gangligerum	10	6.1	5	3.4
C. globosum	5	3	7	4.8
Emericella nidulans	5	3	5	3.4
E. variecolor	2	1.2	2	1.4
Lewia infectoria	5	3	5	3.4
Monascus rubur	–	–	3	3.4
Penicillium citrinum	7	4.2	5	3.4
P. nigricans	5	3	–	–
Fungi imperfecti				
Alternaria alternata	4	2.4	5	3.4
Cladosporium herbarum	2	1.2	5	3.4
Curvularia lunata	10	6	5	3.4
C. verruculosa	2	1.2	–	–
Fusarium oxysporum	8	4.8	10	6.8
Monilia sitophila	5	3	8	5.5
Phoma nebulosa	8	4.8	2	1.4
Trichoderma lignorum	2	1.2	5	3.4
T. viride	5	3	–	–
Sterile white mycelium	10	6	8	5.5
Sterile black mycelium	5	3	5	3.4
Total no. of fungal colonies	165		146	

*Data represent the total number of colonies that appeared on 5 PDA plates after 7 days of incubation; –, absence of colony.

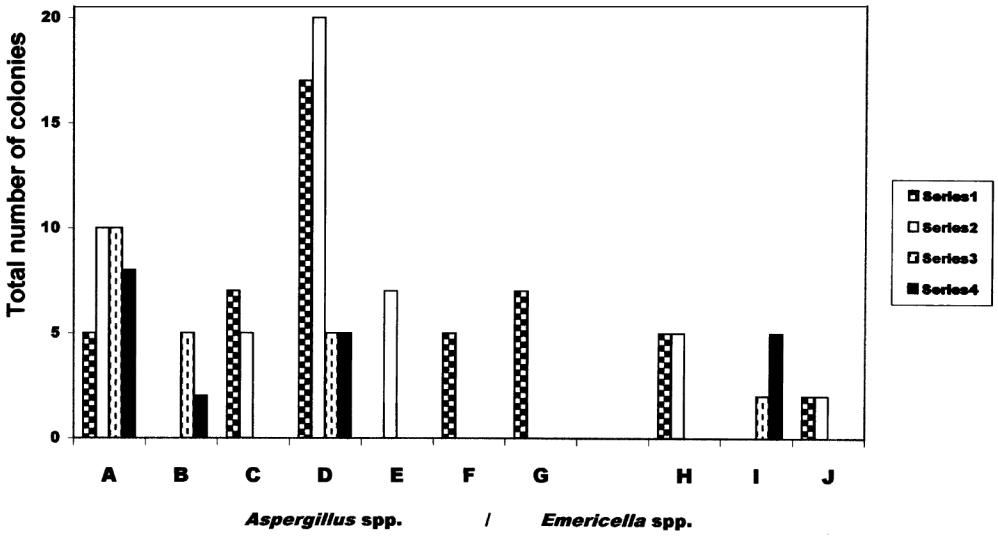

Figure 1. Histogram showing occurrence of *Aspergilli* (*Emericella*) in indoor air of Egypto-Babylonian Gallery and mummy chamber. Series 1 was isolated in 1999, Series 2 in 2000, Series 3 was present in air of the chamber, and Series 4 in dust of the chamber. A, *A. flavus*; B, *A. fumigatus*; C, *A. japonicus*; D, *A. niger*; E, *A. parasiticus*; F, *A. ustus*; G, *A. wentii*; H, *E. nidulans*; I, *E. nivea*; J, *E. variecolor*.

'Mummy' was derived. Herodotus, a much travelled historian and geographer, has given the following account about mummification[21], 'The mode of embalming includes taking out the brain by a crooked piece of iron, cutting the abdomen with an Ethiopian stone, taking out of the whole contents of the abdomen, which they then cleanse, washing it thoroughly with palm wine. The body is filled with purest bruised myrrh, with cassia and every sort of spicery, except frankincense and the opening sewn-up. Then the body is placed in natrum for seventy days and then it is washed and wrapped around from head to foot with bandages of fine linen cloth, smeared over with gum. The beautiful drawings are made to depict the details of the dead person'. The innumerable mummies preserved for thousands of years in mastabas, pyramids, royal tombs, or simply dry earth, prove that Herodotus' account, though it refers to the Late Period, is to some extent applicable to the whole span of Egyptian history. Egyptians felt that heart or *Ib* was the source of intelligence, memory and wisdom. It was also considered to be associated with bravery, sadness and love. So they tried to keep the heart intact, while the other body parts were removed and chemically treated and kept in ornamental urns called the canopic jars. These were placed in the tomb along with the mummy.

The mummy of a lady is placed in Egypto-Babylonian Gallery in a glass chamber of the size of 214 cm × 91.5 cm × 81.5 cm (Figure 3 *a*). There is a wooden dome of height 116 cm at the top of the glass chamber. The size of the mummy kept in the museum is 154 cm × 35 cm × 17 cm (Figure 3 *b*). It has a coffin box made up of wood obtained from Sekamore tree, belonging to family Asclepiadaceae. This mummy belongs to late new empire of the Alexandrian period. At that time, Ptollemy II and his wife, his full sister were the co-rulers. Although oldest known mummies date back to 2500 BC, the period of this mummy has been ascertained to be 230 BC by an Egyptian mummy expert, Nasry Iskander.

It is evident from the Table 1 that 24 species of fungi belonging to 15 genera were isolated in 1999 and 22 species of fungi belonging to 17 genera were isolated in 2000. Occurrence of unidentified non-sporulating white and black mycelial fungi was also recorded. Presence of viable spores of *Aspergillus japonicus*, *A. wentii*, *Chaetomium atrobrunneum*, *C. gangligerum*, *Lewia infectoria*, *Monascus ruber* and *Phoma nebulosa* in indoor air is noteworthy. These biodeteriogens have the potential to cause plant and animal diseases as well as biodeterioration of cultural property. Isolations from exposed petri plates resulted in pure cultures of six *Aspergillus* spp. and two of its teleomorphs, as well as two *Penicillium* spp. Association of *A. versicolor*, *A. terreus*, *Penicillium* sp., *C. gangligerum* and an actinomycetes *Streptomyces griseoflavus*, has been reported with miniature paintings of the Baroda Museum[6]. An earlier study revealed the presence of 22 species belonging to 12 different genera in the Baroda Museum[16]. According to Nair[22], 'Fungi that can grow on any suitable substrata play a prominent role in deterioration of museum materials'. The microbial flora inside and outside the Aurangabad caves as well as Ajanta and Ellora caves were assessed[4,9]. Unlike the present study where culture plate exposure method[19] was used, Rotorod sampler modified by Harrington[23] was employed in these caves to calculate total spore count. Presence of four different fungi belonging to Zygomycotina group was recorded in a sampling done in May 2000. Tilak and Kulkarni[4] and Tilak *et al.*[9] failed to detect spores of such fungi during their surveys. Conidia of Aspergilli and Fungi imperfecti dominated in their slides. They concluded that the excreta of birds and bats residing in these caves serves as a feeding source for the microorganisms and this acts as a secondary source of aerospora.

Isolations made from the air in the mummy chamber revealed the dominance of *Aspergillus flavus*. Other Aspergilli included *A. fumigatus* and *A. niger*. One teleomorph, *E. nivea*, was also observed. Fungal flora of

Table 2. Percentage occurrence of different fungi in air and dust of mummy chamber on 16 April 1999

	Mummy chamber			
	Air		Dust	
Fungi isolated	Colonies*	Percentage occurrence	Colonies*	Percentage occurrence
Aspergillus flavus	10	35.7	8	29.6
A. fumigatus	5	17.8	2	7.4
A. niger	5	17.8	5	18.5
Emericella nivea	2	7.1	5	18.5
Penicillium citrinum	2	7.1	2	7.4
Mucor hiemalis	2	7.1	5	18.5
Unidentified white mycelium	2	7.1	–	–
Total no. of colonies	28		27	

*Data represent the total number of colonies that appeared on 5 PDA plates after 7 days of incubation; –, absence of colony.

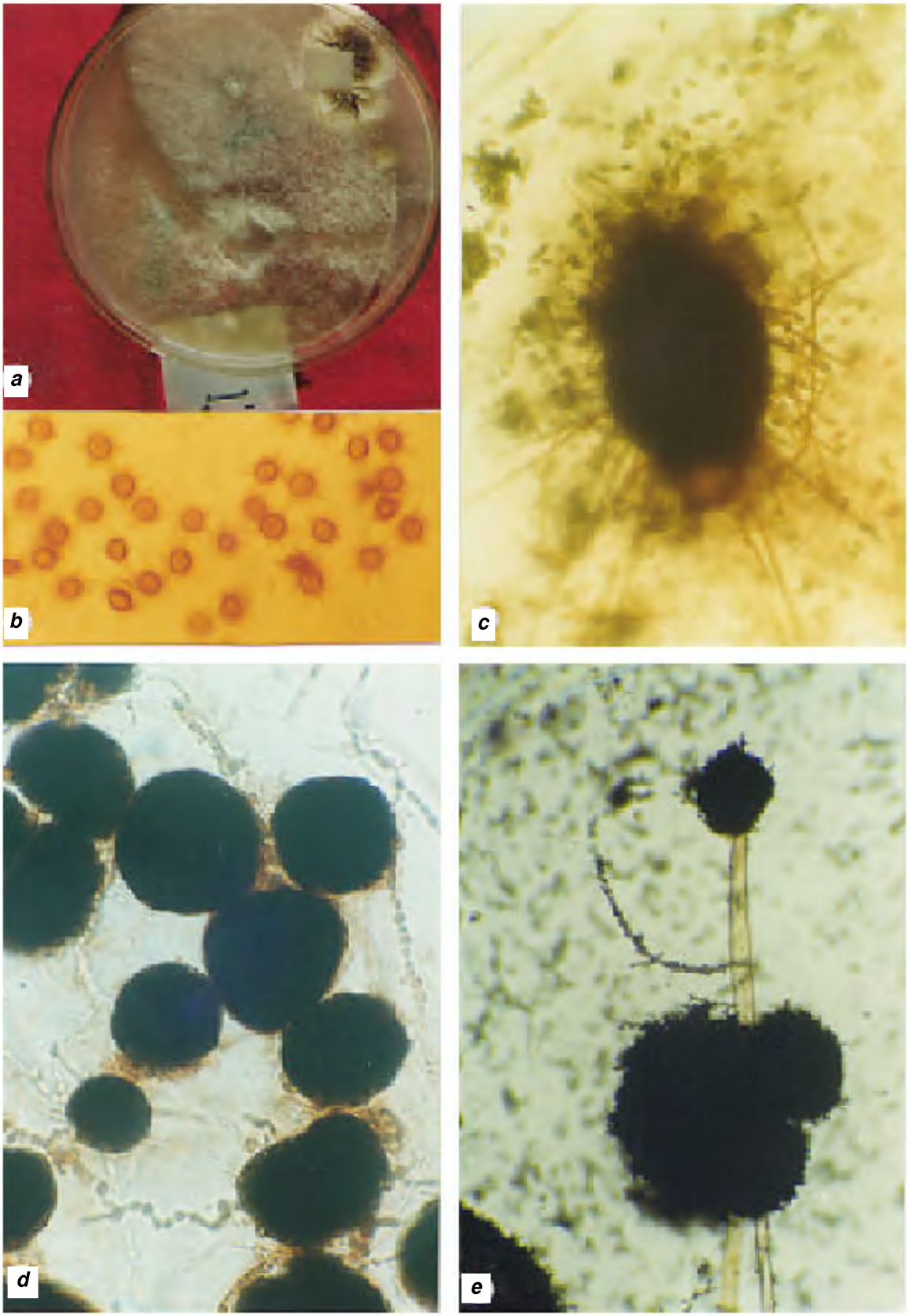

Figure 2. ***a***, Petri dish showing culture of *Aspergillus* isolated from the mummy by cellophane tape technique. ***b–e***, Fungi present in air of the gallery/mummy chamber. ***b***, Photomicrograph showing ascospores of *E. nidulans*, × 940; ***c***, Perithecium of *Chaetomium atrobrunneum* with straight hairs and oval ascospores, × 940; ***d***, Dark spherical cleistothecia of *Monascus ruber* isolated from indoor air of Egyptian Gallery, × 94; ***e***, Conidiophore and conidial heads of *Aspergillus niger*, × 126.

the dust accumulated in the coffin box showed the presence of 6 different members. Along with the four fungi listed above, *Penicillium citrinum* and *Mucor hiemalis* were also recorded. Powdered wood was accumulated in the form of dust in the coffin box. Various slits present in the glass chamber might have allowed the suspended dust particles to settle at the bottom with powdered wood and insect remains.

Aspergillus and *Cladosporium* are two universal biopollutants[22,24]. In a survey of the atmosphere over Jabalpur, 66% of the trapped spores was *Aspergillus* type[25]. Of the 20 species of *Aspergillus* recorded from Lucknow city, 12 were found to be potential allergens[26,27]. Agrawal and Dhawan[28] reported common fungi causing damage to museum objects. *A. tamarii* was found to be associated with the miniature paintings kept in the museum of the Department of Museology of M.S. Univ. of Baroda[29]. *Aspergillus* and *Penicillium* may be considered as the two most serious biodeteriogens, as some of the species can survive for 30–40 years[28]. Cooke and Rayner[30] have described the ecology of saprophytic Aspergilli. According to them, 'Water is required by fungi not only as metabolite and solvent but also to maintain sufficient hydrostatic pressure within hyphae to drive their apical extension'. Unlike field fungi which require water potential to be above – 150 bar, the storage fungi are active between – 500 and – 150 bar. The presence of *A. flavus* in the aerospora of the mummy chamber may be explained by the prevailing hot conditions and water potential exceeding – 220 bar.

In order to solve the curiosity of the visitors, the linen bandage was partly removed from the feet of the mummy by the museum curator fifty years ago. The toes are thus exposed to air. An unusual white-coloured spot was observed two years ago on the first toe of the right leg. After six months, an almost similar but smaller spot was noticed on the third toe (Figure 3 *d*). Now the deterioration has started in this toe also. To confirm the involvement of any fungus, the isolations were made from these spots. The fungal growth was completely removed with the help of 90% ethanol after collecting the samples. Similar spots reappeared after one month. The same fungus was re-isolated on 5 May. On the basis of morphological characters and colour of the colony, the

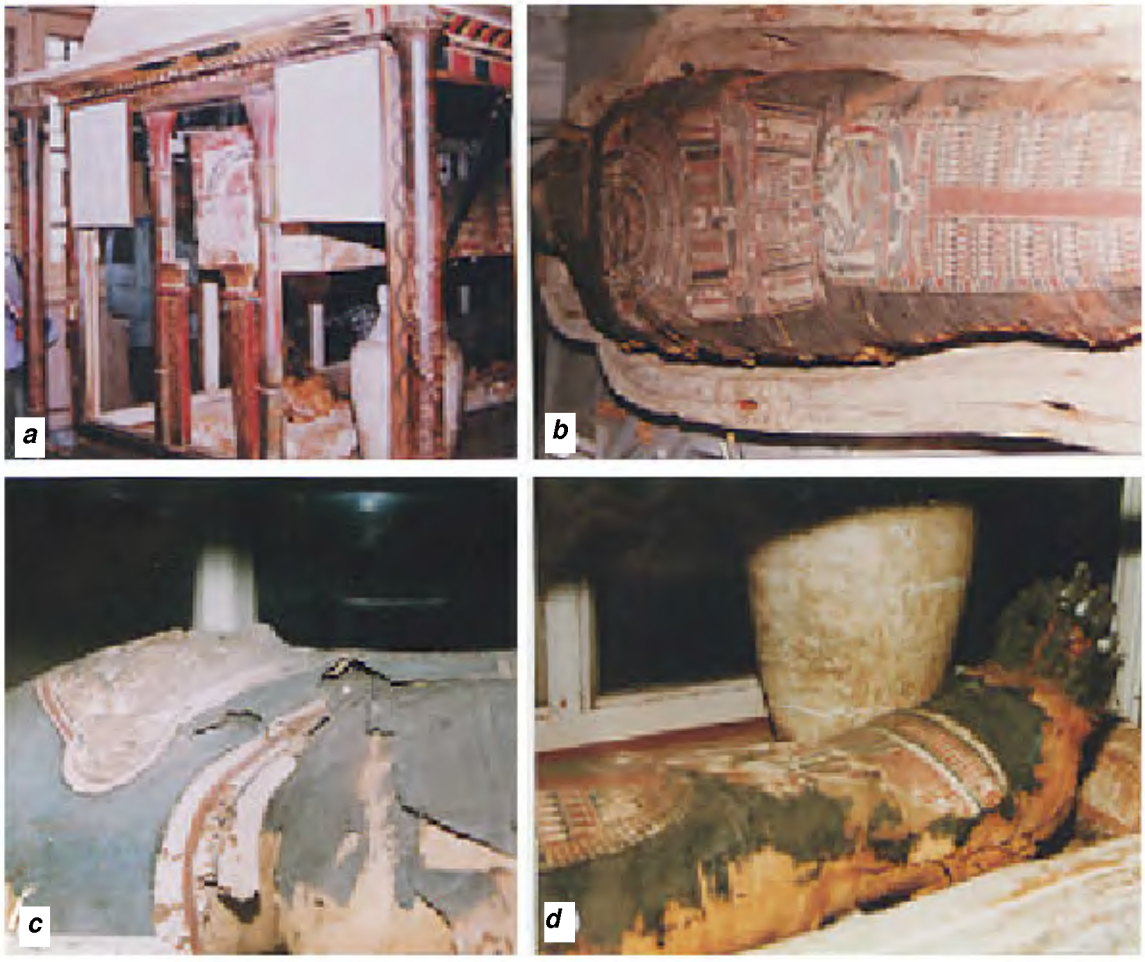

Figure 3. ***a***, Decorated glass chamber in which mycoflora was studied; ***b***, Egyptian mummy kept in coffin box. Note the painting on the outer cover; ***c***, Deteriorated head of the mummy due to activity of insects; ***d***, Lower part of the Egyptian mummy showing white coloured fungal growth on toes. Also visible is the canopic jar.

fungus has been identified as *Emericella nivea* Wiley & Simmons. Agharkar Research Institute, Pune, confirms identity of the fungus.

Colonies of *E. nivea* on Czapek-Dox's agar were white becoming creamy with age. Colonies were slow-growing on PDA slants. Conidiophores measure 600 µm to 1000 µm long, heads with white globose vesicle 20 µm to 30 µm in dia (Figure 4 *a*). Phialides in two series, primary sterigmata 5–8 µm × 2.5–3 µm, secondary sterigmata 5–7 µm × 2–2.5 µm. Conidia colourless 2.2–3.3 µm in size, globose, smooth. Old culture tubes containing PDA slants showed the presence of perfect state. Rounded black cleistothecia were seen in culture. These were 350–400 µm in dia (Figure 4 *b*, *c*). Asci were evanescent, ascospores smooth-walled with a pulley, 4.4–5.5 µm in dia (Figure 4 *d*).

Presence of *E. nivea* in indoor air and on the mummy kept at the Baroda Museum is new to the science. Earlier the anamorph of this fungus, *Aspergillus niveus* Blochwitz was isolated from air in northern Italy at 1700 m[31]. Studies have shown the presence of another species of *Emericella*, *E. nidulans* (Eldam) Vuilll., in the indoor air of different galleries of the Baroda museum[18].

Fungi are major contributors to the deterioration of cultural property in tropical countries like India. During the present study members belonging to Zygomycotina group were also recorded from the mummy chamber and Egypto-Babylonian Gallery. Earlier Tilak *et al.*[9] and Tilak and Kulkarni[4] failed to obtain qualitative data about such Phycomycetes members. It is the drawback of the total spore count method, as spores with identical morphological characters are not clearly identified. Later studies revealed the presence of a number of cellulose-degrading fungi, including *Rhizopus* from Ajanta caves[10]. A perusal of Table 1 clearly indicates that fungal spore diversity was very high in the Baroda museum. There was dominance of the members of Ascomycotina and Fungi imperfecti. Most of the members

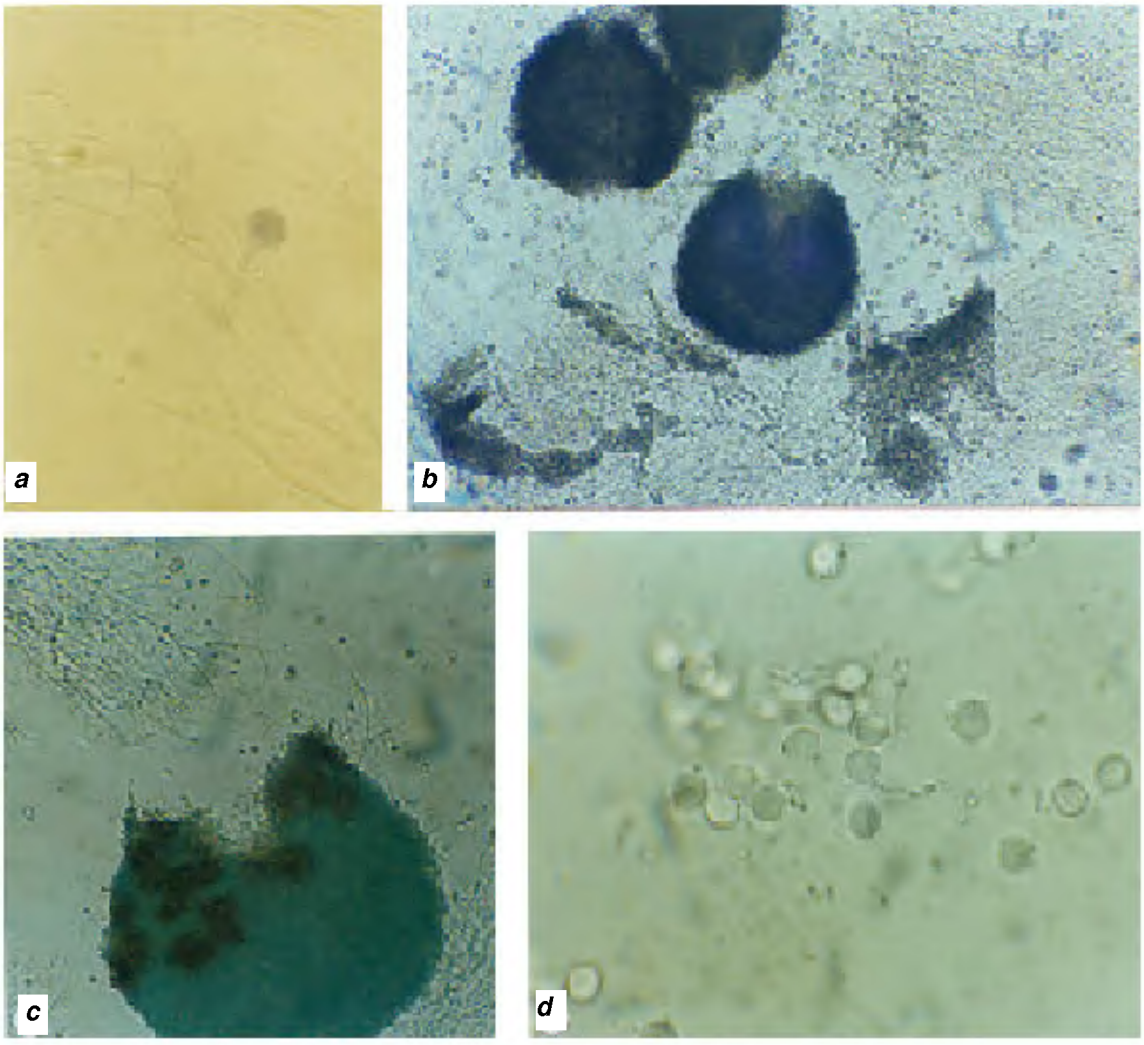

Figure 4. ***a***, *Aspergillus* state of *E. nivea*. Note the conidiophore and conidial head with chains of conidia. This fungus was isolated from fingers of the mummy, × 940; ***b***, Cleistothecia and ascospores of *E. nivea*, × 94; ***c***, Fungal hyphae and dark-coloured single broken cleistothecium, × 94; ***d***, Photomicrograph showing ascospores of *E. nivea*, a fungal biodeteriogen, × 940.

of the Fungi imperfecti group were phytopathogens. Their occurrence may be correlated with the surrounding environment which is full of plants. Efforts are on to study the deteriorating potential of *E. nivea* on museum materials and to suggest effective control measures.

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Phenology of understorey species of tropical moist forest of Western Ghats region of Uttara Kannada district in South India

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Vegetative and reproductive phenology of 107 (52 shrubs, 8 liana, 11 climbers and 36 herb species) understorey species of tropical moist forests of Uttara Kannada district in the Western Ghats of South India was monitored from November 1983 to December 1985, through fortnightly visits to eight one-hectare sites. A prominent peak in leaf flush, flowering and fruiting occurred in the pre-monsoon period in shrubs and lianas, while leaf abscission occurred during the post-monsoon winter period. In the climbers and herbs, flowering and fruiting were concentrated in a single peak during the post-monsoon period. Leaf flush and flowering in deep-rooted shrubs and lianas may have been triggered by changes in day-length and temperature; moisture availability may govern these events in the shallow-rooted climbers and herbs. It is argued that moisture availability, herbivore, pollinator and disperser abundance may have shaped the phenological patterns of the species in these forests.

TROPICAL plant communities with their high levels of species diversity display phenological events staggered in time and space[1,2]. Understanding of such behaviour of the communities is useful in evolving proper management

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