Mycopath (2004), 2(1): 43-50

Mycoflora associated with the biodeterioration of picture walls at Lahore Fort

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Abstract

A total of 25 fungal species belonging to 10 genera were found associated with biodeteriorating picture walls at Lahore fort. These included 13 species of *Aspergillus*, two species each of *Alternaria*, *Drechslera* and *Fusarium*, and one each of *Acremonium*, *Curvularia*, *Helminthosporium*, *Mucor*, *Trichoderma*, and *Dematium*. *Alternaria alternata*, *Aspergillus niger*, *A. flavus* and *A. fumigatus* were highly frequent and apparently major cause of biodeterioration. No much pronounced variation in fungal flora among the selected sites was evidenced. A great variation in variety of the fungal flora was, however, evident on 7 different type of growth media employed viz. corn meal dextrose agar (CMDA), Czapek's dox agar (CZA), oat meal agar (OA), malt extract agar (MEA), potato dextrose agar (PDA), rose bengal agar (RBA) and sabouraud's dextrose agar (SDA). Maximum fungal colony count was observed on CZA while highest fungal diversity was recorded on MEA. The fungal flora was isolated by two methods namely tape plate and scratch method, the later method appeared to be more reliable than the former.

Introduction

The city of Lahore is undoubtedly an ancient and one of the cultural, architectural and artistic centers of the country. It possesses magnificent remnant and testimonial of brilliant Mughal civilizations among which Palace, the Lahore Fort stands out prominently as an Islamic medieval architecture and speaks eloquently of centuries of passing history.

Among the large variety of Islamic Architectural masterpieces in Lahore Fort, the tradition of picture walls is very old, dating back to prehistoric time. These picture walls, which are valuable not only as work of art but also as a source of invaluable information about Mughal history, occupy a place of pride in the repository of nation's cultural heritage, hence hardly need any justification for its preservation. Due to continuous neglect to its maintenance efforts, the process of spoilage and mutilation had gone on for well over a century. Then in 1927, the fort was taken over by Archeological Survey to maintain it as a historic monument. Presently, the fort is under severe damage, losing its dignity and identity very rapidly by adverse deterioration and decay caused by different physical, chemical and biological processes. On the way toward extinction of different materials, picture walls are at the worst, being most susceptible to microbial attack.

A broad spectrum of organisms such as algae, bacteria, insects and fungi are involved in the deterioration of various types of material such as mortars, plasters, frescoes and ceramic products. Fungi, however, are the major group of biodeteriorants and are responsible to a large extent for decay of murals. It is due to higher fungal growth under optimum humid environment and temperature conditions and availability of nutrients on picture walls. The problem of deterioration by fungi is worldwide and deterioration of this work of art has been reported from different countries like Iraq, Japan, Spain, England, Russia, Germany, Bulgaria, Italy, Romania, Poland, Switzerland, India and some other countries of the world (Krumbein and Lange, 1978; Jeffries, 1986; Agrawal *et al.*, 1991; Rebricova, 1991; Garg *et al.*, 1993).

The present study, therefore, was designed to investigate the occurrence of biodiversity of fungal flora associated with the picture walls of Lahore fort on different types of culture media. It will bring about a vivid picture of fungi on picture walls into focus, responsible for severe deterioration and decay.

Material and Methods

Fungal deterioration studies were carried out on picture walls of Lahore Fort (Plate 1). Ten sites were selected for collection of fungal flora involved in biodeterioration. Sampling material was scratched with the help of sterilized scalpel and etched with sticking tape from the walls. As different fungal species prefer to grow on different culture media, so to maximize the spectrum of

investigation and to capture all fungal agents of deterioration, a variety of culture media were employed viz. CMDA, CZA, MEA, OA, PDA, SDA and RBA. Inoculated petriplates were maintained at 25±1ºC for 7 days. Each sample was replicated three times. After incubation period fungal colonies were counted, calculated and identified. Data was analyzed statistically by applying Duncan's New Multiple Range Test (Steel and Torrie, 1980).

Results

The intensive culture studies of saprophytic fungi associated with biodeterioration of picture walls at Lahore Fort from 10 different sampling sites lead to isolation of 25 species of fungi belonging to 10 genera. The various species that appeared on specific and wide spectrum media included 13 species of *Aspergillus*, 2 each of *Alternaria* , *Drechslera* and *Fusarium* and 1 each of *Acremonium, Curvularia, Dematium, Helminthosporium, Mucor* and *Trichoderma.*

Table 1: % frequency of occurrence of various fungal species

Sr. #	Name of fungal species	Total colonies	% occurr-ence
1.	*Acremonium sp.*	15	1.25
2.	*Alternaria alternate*	150	12.53
3.	*Alternaria sp.*	31	2.58
4.	*Aspergillus alutaceus*	16	1.33
5.	*A. japonicus*	15	1.25
6.	*A. carneus*	19	1.41
7.	*A. flavus*	150	12.52
8.	*A. flavipus*	15	1.52
9.	*A. fumigatus*	175	14.60
10.	*A. melleus*	16	1.33
11.	*A. nidulans*	36	3.00
12.	*A. niger*	213	17.78
13.	*A. oryzae*	10	0.83
14.	*A. terreus*	13	1.08
15.	*A. ustus*	12	1.00
16.	*A. wentii*	12	1.00
17.	*Curvularia lunata*	50	4.17
18.	*Dematium sp.*	16	1.33
19.	*Drechslera australiensis*	105	8.76
20.	*D. tetramera*	13	1.08
21.	*Helmenthosporium sp.*	52	4.34
22.	*Fusarium moniliforme*	13	1.08
23.	*Fusarium oxysporum*	17	1.41
24.	*Trichoderma sp.*	52	4.34
25.	*Mucor sp.*	11	0.91
	Grand Total	**1198**	

Table 2: % occurrence of fungal colonies on different media

Sr. #	Name of fungal species	Total colonies	% occurrence
1.	corn meal dextrose agar	152	12.68
2.	Czapek's dox agar	254	21.02
3.	malt extract agar	207	17.27
4.	oat meal agar	132	11.01
5.	potato dextrose agar	158	13.18
6.	rose bengal agar	126	10.51
7.	Sabouraud's dextrose agar	169	14.01
	Total colony count	**1198**	**99.86**

Fungal species composition on picture walls

The spectrum of species found associated with picture walls at Lahore Fort are presented in (Table 1). *Aspergillus niger* van Tieghem, *was* ranked as the most prevalent species significantly with greater percentage occurrence with a colony count of 17.72% followed by *Aspergillus fumigatus* Fres (14.60%), *Alternaria alternata* (Fr.) Keissler, (12.41%), *Aspergillus flavus* Link ex Gray (12.52%), *Drechslera australiensis* (Bugnicourt) Subram. & Jain ex M.B. Ellis (8.76%). The less common species were *Helminthosporium* sp. (4.34%), *Curvularia lunata* (Wakker) Boedjin (4.17%), *Alternaria sp.* (2.58%), *Aspergillus nidulans* (Eidam) Winter (3.00%), *Mucor* sp. (1.91%), *Acremonium* sp. (1.25%), *Aspergillus japonicus* Saito (1.33%), *A. alutaceus* Bark. & Curt. (1.25%), *A. carneus* Blochwitz (1.41%), *A. flavipus* (1.25%), *A. melleus* Yukaawa (1.33%), *A. oryzae* (Ahlburg) Cohn (0.83%), *A. terreus* Thom (1.08%), *A. ustus* (Bain.) Thom & Church (1.00%), *A. wentii* Wehmer (1.00%), *Dematium* sp. (1.33%), *Drechslera tetramera* (Mckinney) Subram & Jain (1.08%), *Fusarium moniliforme* Sheld. (1.08%) and *F. oxysporum* Schllecht (1.14%) (Fig. 1).

Effect of growth media on fungal population

Marked variation in colony count of different fungi was observed on different growth media. A total of 1198 colonies were recorded. The results indicated that best nutrition was provided for growth of these fungi by CZA which supported a significantly greater number of fungal colonies (254) followed by MEA (207). Colony count obtained on other media i.e. SDA, PDA and CMDA was 169, 158, and 152, respectively (Table 2).The number of fungal colonies was significantly low on OA (132) and RBA (126) as compared to other media, exhibiting poor quality of nutritional supplements (Fig. 2).

MEA proved best for fungal growth and supported maximum type of genera (10) and species (23), while minimum number.i.e.4 genera and 7 species appeared on RBA (Fig. 3).

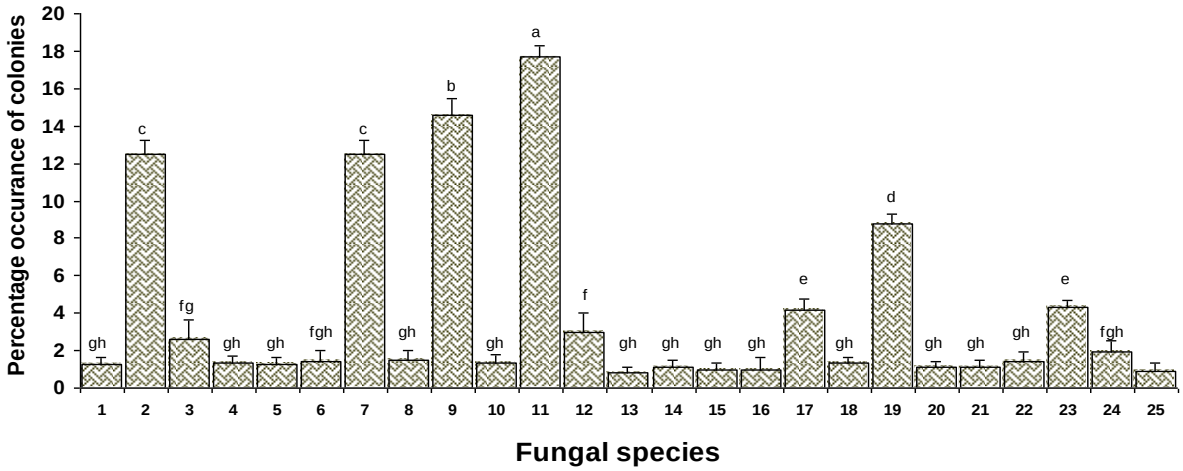

Fig. 1: Mycoflora isolated from different sampling sites of picture walls of Lahore Fort.

1-*Acremonium* sp.; 2-*Alternaria alternata;* 3-*A. sp.;* 4-*Aspergillus alutaceus;* 5-*A. japonicus;* 6-*A. carneus;* 7-*A. flavus;* 8-*A. flavipus;* 9-*A. fumigatus;* 10-*A. melleus;* 11-*A. niger;* 12-*A. nidulans;* 13-*A. oryzae;* 14-*A. terreus;* 15-*A. ustus;* 16-*A. wentii;* 17-*Curvularia lunatus;* 18-*Demaitum* sp.; 19-*Drechslera austrialensis;* 20-*D. tetramera;* 21-*Fusarium moniliforme;* 22-*Fusarium oxysporum;* 23-*Helminthosporium* sp.; 24-*Trichoderma* sp.; 25-*Mucor* sp.

Vertical bars show standard errors of means of 10 sites.

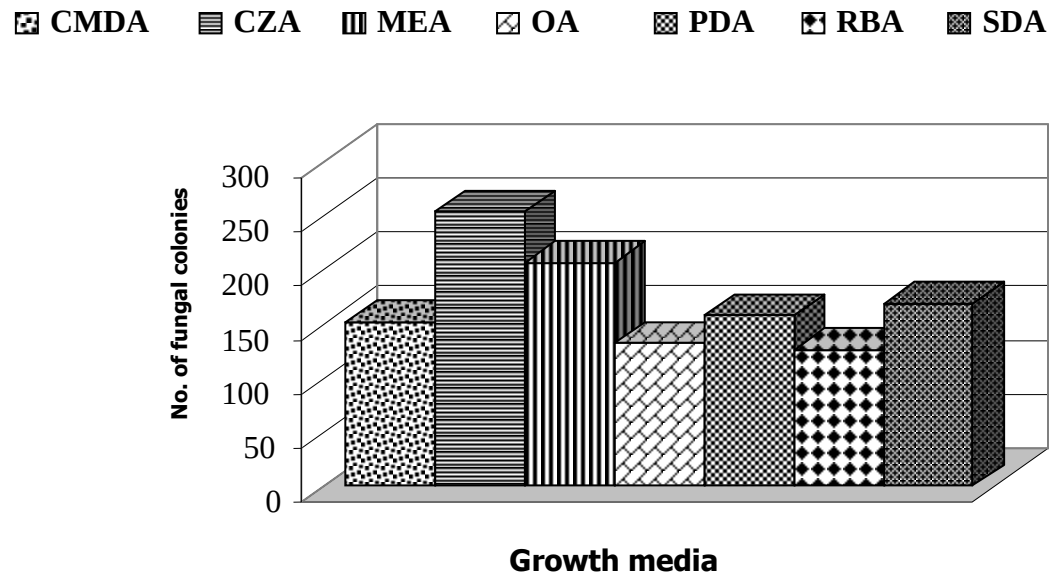

Fig. 2: Effect of growth media on number of fungal colonies

CMDA: corn meal dextrose agar, CZA: Czapek's dox agar, MEA: malt extract agar, OA: oat meal gar, PDA: potato dextrose agar, RBA: rose bengal agar, SDA: Sabouraud's dextrose agar

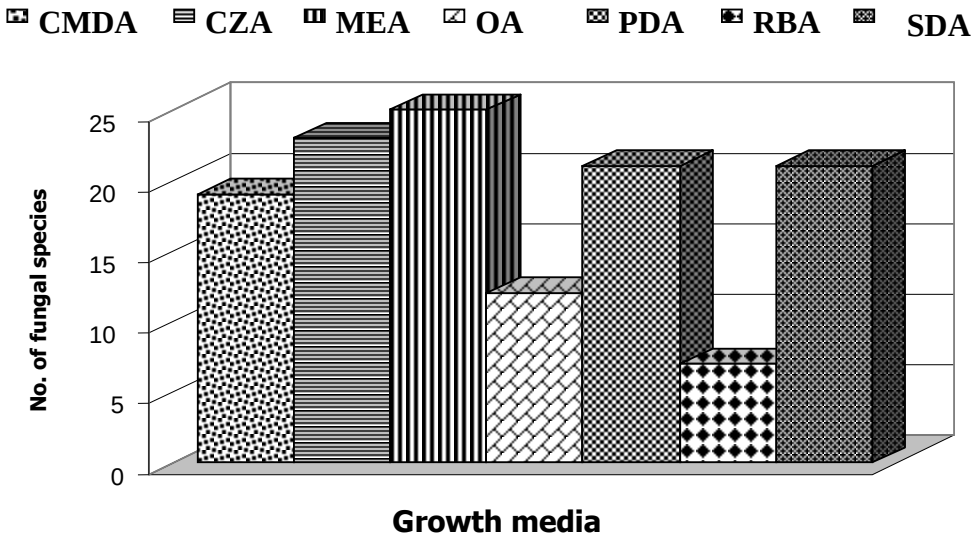

Fig: 3: Effect of growth media on number of fungal species

CMDA: corn meal dextrose agar, CZA: Czapek's dox agar, MEA: malt extract agar, OA: oat meal gar, PDA: potato dextrose agar, RBA: rose bengal agar, SDA: Sabouraud's dextrose agar

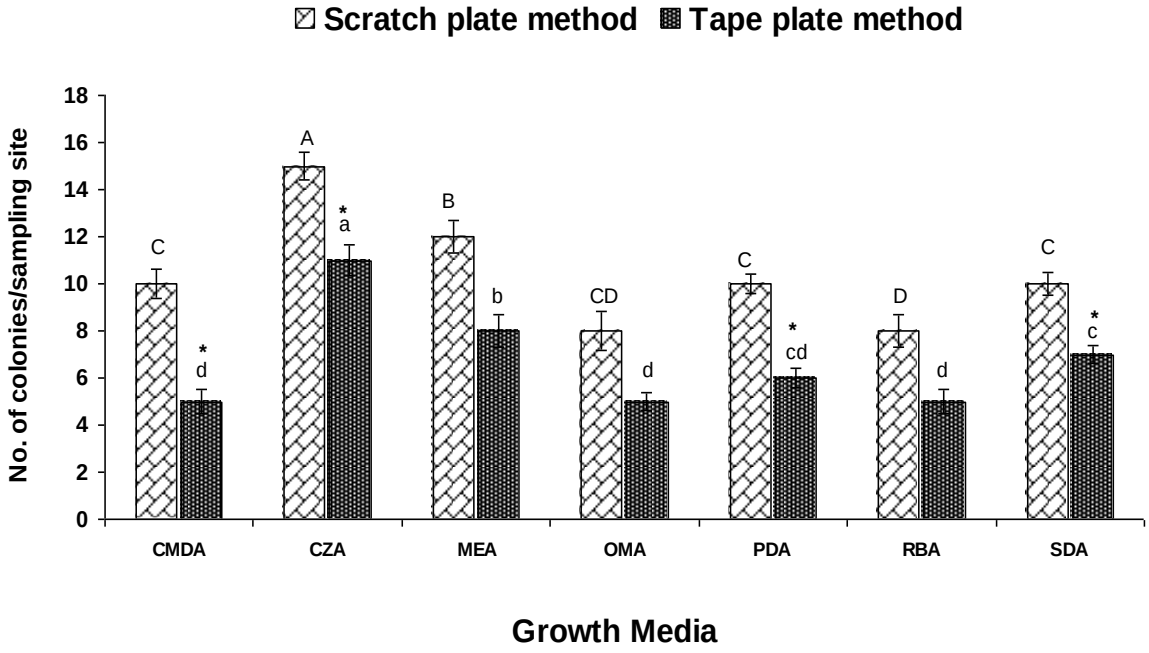

Fig. 4: Comparison of sampling techniques

CMDA: Corn meal dextrose agar, CZA: Czapek's dox agar, MEA: Malt extract agar, OA: Oat meal gar, PDA: Potato dextrose agar, RBA: Rose bengal agar, SDA: Sabouraud's dextrose agar

Values with different letters in upper and lower cases show significant difference between the treatments in scratch plate and tape plate method, respectively, as determined by DMR test.

*, show significant difference (P = 0.05) between scratch plate and tape plate method as determined by t-test.

ns: non-significant.

Plate 1: Extent of decay at various sampling sites of Picture walls at Lahore Fort
a: peeling off, b: discoloration, c: staining, d:cracking

Influence of sampling techniques

Two different sampling techniques, i.e., scratch plate method & tape plate method were employed during this investigation. Statistical analysis clearly showed a significant (P = 0.05) difference in terms of colony count among these two plating methods. A great diversity of fungal species composition with maximum colony number was obtained by scratch plate method than tape plate method on different type of media employed (Fig.4).

Discussion

The results of present investigation revealed a fairly advanced stage of biodeterioration of picture walls at Lahore fort. A large number of fungal deteriorants were found to be associated with this process .The isolations lead to a total of 1198 fungal colonies comprising of 10 genera and 25 species on variously employed growth media.

Species of *Aspergillus, Alternaria, Curvularia, Drechslera, Mucor, Fusarium, Acremonium, Trichoderma, Helminthosporium* and *Dematium* were found to be prevalent during the current studies. These findings are in line with earlier studies carried out globally by several workers (Hirte *et al*, 1987; Rebricova, 1991; Garg & Dhawan, 1994). However, the genera like *Helminthsporium* and *Dematium* have not been reported in former investigations. Similarly, *Aschersonia, Chaetomium, Cladosporium, Emericella, Macrophomina, Rhizopus, Stachybotrys, Paecilomyces, Penicillium Sporotrichum, Epicoccum, Stemphylium, Torula, Bispora, Nigrospora* and *Pithomyces* reported by former authors were not found in association with picture walls in this study. The variations in species composition of deteriorants can be attributed to varying physical and climatic factors over geographically widely distributed countries of the world.

During present study species of *Aspergillus* and *Alternaria* exhibited maximum percentage of occurrence, while the species of *Acremonium, Curvularia, Dematium, Drechslera, Fusarium, Helminthsporium, Mucor* and *Trichoderma* showed low percentage of occurrence. The species of *Aspergillus* and *Alternaria* as major decay agent shave also been observed in previous studies from Pakistan and India to be the most prevalent air borne genera by Nayyera (1971); Gaur (1980) and Talpur *et al*., (1995). As Lahore Fort is situated in thickly populated localities with high levels of air pollution, presence of these two genera is obvious as major air borne biodeteriorants on picture walls of Lahore Fort as same has been reported previously by Shah (1995) as most prevalent air born genera. Relatively low percentage occurrence of *Mucor, Fusarium, Curvularia, Dematium* and *Helminthosporium,* however is not in agreement with the findings of Nayyara (1971); Bajwa *et al.,* (1995b) and Shah (1995), who found these genera as dominant component of aeromycoflora of Lahore. The fast environmental changes (Adeeb and Baig (1995) during the last decades or so may have led to cause appearance of some species and disappearance of others.

The various constituents of picture walls undergo deterioration physically, chemically and biologically (Mora *et al*., 1984). As a consequence of deterioration by fungal growth on picture walls of Lahore Fort, two types of aesthetic damages were observed i.e. pigment discoloration and staining (white and green-to-black stains) whereas, structural damages were cracking and disintegration of surface layers. Similar damages have also been reported by Hyvert (1966); Ionita (1971) and Andersson *et al.* (1997) from both types of alterations and reported species of *Cladosporium, Aspergillus, Acremonium, Penicillium, Helminthosporium* and *Alternaria* within stone and other historic building materials. Krumbein and Lange, (1978) and Strzelezyk (1981) found the species of *Aspergillus, Fusarium* and *Mucor* to be the most active deteriorants, responsible for darkening and disfiguring of exterior surface of buildings. According to Leznicka *et al.* (1988) various stains are due to melanins (very stable indochinone derivatives) present inside the mycelium. The mycelium can penetrate deeply inside the plaster of wall and causes loss of cohesion and detachment of the layers.

In order to trap the maximum number and diversity of fungal species from picture walls seven different types of media were used viz. CMDA, CZA, MEA, OA, PDA, RBA, and SDA. In present study maximum number of fungal colonies were isolated on CZA, which is not in line with previous findings of Bajwa *et al.*, (1995a), Bajwa *et al*., (1995b) and Shah (1995), which found maximum diversity of aeromycoflora on MEA, as also reported by Woollenzien *et al*., (1995). MEA supported greatest diversity being a better source of nutrient for large spectrum of fungal species in general followed by on PDA. This attribute also gets support from earlier investigations of Diakumaku *et al.,* (1995). Number of fungal colonies and fungal diversity was also higher on SDA and large number of fungal colonies with less diversity was found growing on CMDA. OA and RBA allowed restricted growth of only specific species of fungi.

The adhesion of fungi to the substrate is very important. Indeed, the ability to transform or deteriorate the substrate is strictly linked to good attachment of fungi by their hyphae. Therefore, fungal flora was isolated from picture walls by two methods i.e. tape plate method and scratch plate method. The former techniques have been used by Ciferri (1999) and Bajwa *et al*. (1995a) during isolation studies from works of art with excellent results. During present study greater number of fungal species was obtained by scratch plate rather than by tape plate method. It may be due to the fact that in tape plate method only superficial fungal flora is trapped while in scratch plate method all the fungal species present on or in the picture walls were obtained. The present study indicates that a large number of fungi, majority of which are very aggressive biodeteriorants, are associated with picture walls at Lahore fort. Therefore, Archaeological Conservation Authorities may have to adopt necessary measures probably in coordinated efforts involving bioscientists of the country to check the decay so as to conserve and maintain this significant specimen of cultural heritage for the perusal of the present generation as well as for the benefit of posterity to enjoy and take pride in the contribution made by the Mughal Emperors in this regard.

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