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Research Article

ISOLATION AND MORPHOLOGICAL IDENTIFICATION OF FUNGI FROM DETERIORATING MONUMENTS OF MADHYA PRADESH, INDIA

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ABSTRACT

Fungi are the most commonly occurring microbes found on the surfaces of stone. They have very simple nutritional requirements and can absorb organic compounds from the environment. Fungal hyphae penetrate surfaces and grow inside the soil. This investigation was carried out in order to determine the diverse community of fungi growing on these deteriorating monuments. For this study, 55 samples of stone were collected from 10 ancient sites which were deteriorating. The samples were collected by use of scalpels, brushes, tweezers and stored in UV sterilised poly bags. The isolation was done by using dilution plate method, plating technique and moist chamber method. During this study, a number of fungal species were isolated. These mixed colonies were then purified from the isolation plates and separately incubated at 28±2°C. For this, selective media SDA (Sabouraud Dextrose Agar) was used. In order to prevent contamination from bacterial colonies, Chloramphenicol was added. After isolation, identification was done. For the microscopic examination of fungi compound light microscope was used. Tease mount method was employed to observe the septation, conidia or spores. For the preparation of mount Lactophenol Cotton Blue was used. Twenty- One fungal species were isolated from 17 genera which are accounted in this study. The most frequently occurring fungal species are *Alternaria sp, A. nidulans, A. fumigatus, A. flavus, A. terreus, A. niger, Bipolaris sp., Curvularia sp., Cladosporium sp., Chaetomium sp., Chrysosporium sp., Exserohilum sp., Fusarium sp., Penicillium sp. , Phoma sp., Poecilomyces sp., Talaromyces sp., Trichoderma sp., Torula sp., Ulocladium sp.* and *Verticillium sp.* From the selected sites, there were few sites like Raisen Fort Raisen, Rajwada Palace Indore, Bhimbetka caves Raisen, Islamnagar Fort Bhopal and Bir Singh Palace Datia showed the maximum number of fungal species. These were followed by Shaukat Mahal Bhopal, Sadar Manzil Bhopal, Siddhawat ghat Ujjain, Bharthari Caves Ujjain and Gohar Mahal Bhopal. From these sites, areas showing fungal abundance were chosen for the collection of samples. The bio-corrosion caused by these isolates will help to evaluate the damage caused and their extent of involvement. The data and results brought about from this study will help for future researches and analysis. Moreover, this study determines the vast fungal diversity found on the deteriorating surface of the monuments of Madhya Pradesh. This study will further help in implementing the preventive measures for the conservation of heritage monuments.

Keywords: Deteriorate, Monuments, Fungi, Stone, Surface, Environment, Damage.

1. INTRODUCTION

Madhya Pradesh is popularly known as the “Heart of India”. It is famous for its archaeological heritages, historic monuments and sculptures. There are several forts and Palaces built hundreds of year back by famous rulers of their time. Those sites have now become tourist attraction. The prehistoric monuments are exposed from a very great amount of time. This has caused its stone surface to deteriorate and has caused change in their character. Along with environmental factors, several chemical and biological agents are also responsible for these changes [1]. Microorganisms are foremost among the biological damage to these monuments [2]. Microbial growth has been proved on a variety of surfaces like sandstone, granite, marble, glasses and metals [3]. Several species of bacteria, fungi, moulds, algae, cyanobacteria have been ascertained by scientists in their previous researches [4]. Biodeterioration of historic monuments by fungal species is a major aim for this study.

Fungi are chemo-heterotrophic organisms, *i.e.* they lack chlorophyll and the capability to manufacture their own food [6]. The presence of organic remains on the surface of stone favours the fungal growth [7]. Fungi with massive enzymatic potential and their ability to grow in low water activity have led them to deteriorate the cultural heritage [8]. The initial penetration is done by fungi into the holes or cracks of stone material, which then favours the growth of several bacterial species also [9]. The consequence of these combined actions is causing dwindling and scaling of stone material [10]. The *Fusarium sp.* of fungi was found to be responsible for the release of calcium and weight loss from limestone [11]. Filamentous fungi *Penicillum frequentens* and *Cladosporium cladosporoides* caused deterioration by excreting organic acid on the stone surface [12, 13]. The filamentous fungi deteriorate marble, limestone, granite and basalt rock through the action of excreted acids as oxalic and citric acids [14]. It is reported that basic rocks are more susceptible to fungal attack than acidic rocks [15]. It has also been shown in the laboratory that fungi such as *Aspergillus niger* were able to solubilise powdered stone and chelate various minerals in a rich glucose medium because they produce organic acids such as gluconic, citric, and oxalic acids [16]. It was found that the fungal genera *Cladosporium, Penicillum, Trichoderma, Fusarium* and *Phoma* are most predominant on the monuments [17]. Some fungi are capable of converting the stone into powdered form by their action [18]. Air pollution, rain, animal excretions and human actions are several other factors that contribute to the growth of fungi on the rocks providing low nutrient conditions [19]. Biofilms are developed on the surface of stone due to the constant exposure of harsh environmental conditions [20]. The proposed study will help to find out the detail about fungal community associated with heritage of Madhya Pradesh so that their role in deterioration of these monuments can be studied further. In the present study, various methods have been used to quantify the fungi involved in the biodeterioration processes [21]. This study will help to develop restoration strategies further by the knowledge of fungal diversity found on these monuments [22].

2. MATERIAL AND METHODS

From the selected sites, there were few sites like Raisen Fort Raisen, Rajwada Palace Indore, Bhimbetka caves Raisen, Islamnagar Fort Bhopal and Bir Singh Palace Datia showed the maximum number of fungal species. These were followed by Shaukat Mahal Bhopal, Sadar Manzil Bhopal, Siddhawat ghat Ujjain, Bharthari Caves Ujjain and Gohar Mahal Bhopal. From these sites, areas showing fungal abundance were chosen for the collection of samples (Fig 1).

Following table depicts the ten sites were selected for research

Table 1: Sites Selected for Research from Madhya Pradesh

S. No.	Monument selected	City where monument is located
1.	Raisen Fort	Raisen
2.	Rajwada Palace	Indore
3.	Bhimbetka caves	Raisen
4.	Islamnagar Fort	Bhopal
5.	Bir Singh Palace	Datia
6.	Shaukat Mahal	Bhopal
7.	Sadar Manzil	Bhopal
8.	Siddhawat ghat	Ujjain
9.	Bharthari Caves	Ujjain
10.	Gohar Mahal	Bhopal

2.1. Sample collection and processing of samples

The stone samples were collected from 10 different ancient sites of Madhya Pradesh (Table 1). The samples were collected with the help of sterile scalpel, brushes and tweezers. They were then placed in air tight UV sterilised poly bags. Out of all these ancient sites, the area showing visible microbial growth and patina were selected for sample collection. The samples were then processed by dilution plate technique, plating technique and moist chamber method [23]. Serial dilutions of the samples were prepared by taking 1gm of sample and placing it in 10 ml of sterile distilled water in a tube and shaken by vortexing the mixture for 30 minutes at room temperature. Then 1 ml of first tube was added into 9 ml of sterile distilled water tube, 0.1 ml final dilution was inoculated on selective media SDA (Sabouraud Dextrose Agar) by spread plate technique [24]. Chloramphenicol was added to the molten medium after autoclaving so as to avoid unwanted growth in the medium. One gram of stone sample from each selected site was placed in the centre of plate and autoclaved media was poured by employing the Plating technique. In moist chamber method, the stone samples were sterilised by using UV radiation and the placed in plastic containers. To act as moisture reservoir in the moist chamber, cotton plugs were soaked in double distilled water and placed in the chamber.

2.2. Isolation of Fungi

The plates were incubated at 28±2°C for 4-6 days and carefully examined regularly. Also the moist chambers were incubated at 26±2°C for 21 days and the chambers were kept in dark. After the incubation, fungal hyphae growing on the stone sample were isolated with the help of sterile needle and were cultured on SDA plates. Mixed colonies were obtained from the plates made by these methods (Fig. 2). Fungi were purified by single spore method [25]. By proper distinguishing, single colonies were then further separately grown by placing them in the middle of the plate and incubating at 28±2°C for 4-6 days (Fig. 3). The isolates thus obtained were then subcultured in slants (Fig. 4).

2.3. Identification of fungi

The morphology of isolated fungi was identified by microscopic examination. For this, Tease mount method was employed. By using needles, fragments of mycelia were teased and a mount was prepared by using Lactophenol cotton blue for observing the septation, hyphal structures and conidia or spores. The cultures were examined on the basis of macroscopic features of colonies grown on agar plates, including growth pattern, colony appearance & reverse, colony texture, pigmentation of the colonies on SDA [26] (Fig. 5).

Fig. 1(1): Raisen Fort, Raisen

Fig. 1(2): Rajwada Palace, Indore

Fig. 1(3): Bhimbetka Caves, Raisen

Fig. 1(4): Islamnagar Fort, Bhopal

Fig. 1(5): Bir Singh Palace, Datia

Fig. 1(6): Shaukat Mahal, Bhopal

Fig. 1(7): Sadar Manzil, Bhopal

Fig. 1(8): Siddhawat Ghat, Ujjain

Fig. 1(9): Bharthari Caves, Ujjain

Fig. 1(10): Gohar Mahal, Bhopal

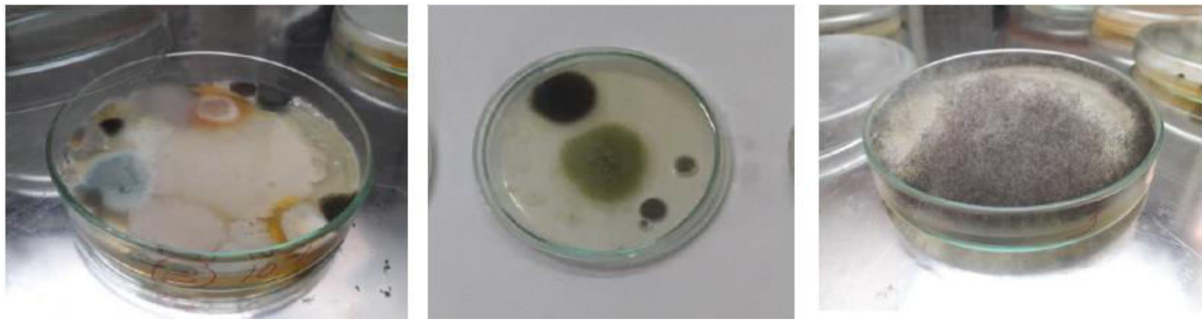

Fig. 2: Mixed colonies obtained from different isolation methods

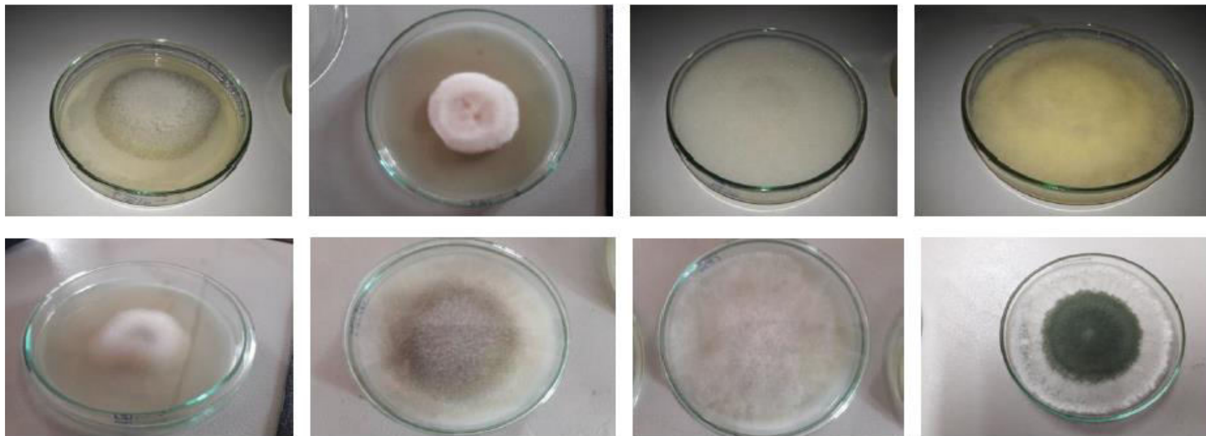

Fig. 3: Fungi isolated from mixed colonies

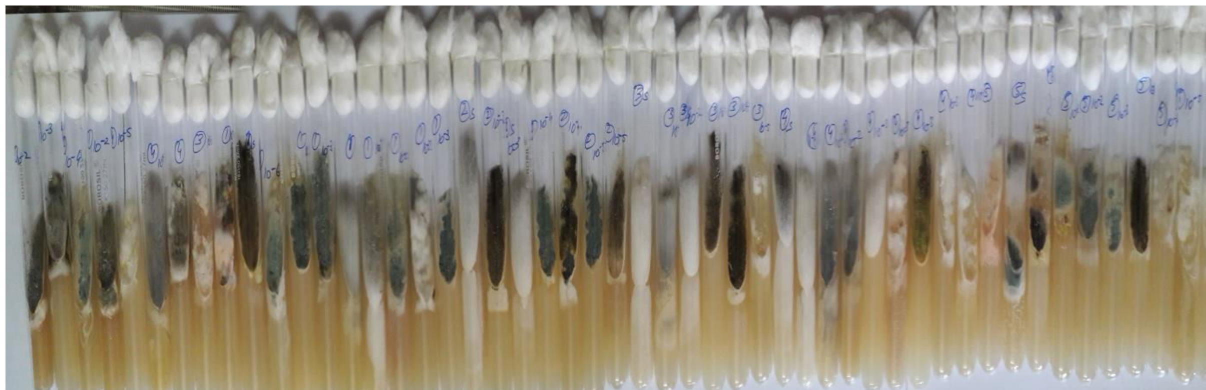

Fig. 4: Fungal isolates obtained from different sites

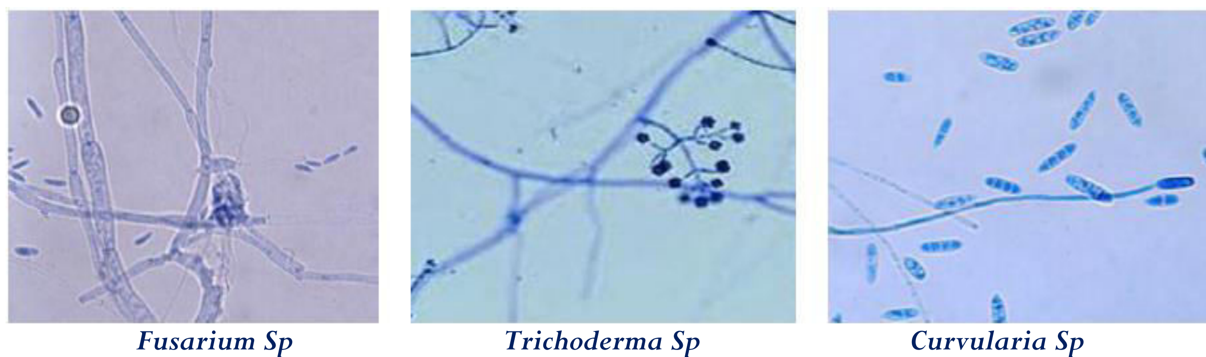

Fig. 5: Identification of fungus by tease mount method

3. RESULTS

To attain the fungal diversity, 55 samples from 10 different sites of Madhya Pradesh were collected and processed. Twenty- one fungal species were isolated from 17 genera which are accounted in this study. The most frequently occurring fungal species are *Alternaria sp, A. nidulans, A. fumigatus, A. flavus, A. terreus, A. niger, Bipolaris sp., Curvularia sp., Cladosporium sp., Chaetomium sp., Crysosporium sp., Exserohilum sp., Fusarium sp., Penicillium sp., Phoma sp., Poecilomyces sp., Talaromyces sp., Trichoderma sp., Torula sp., Ulocladium sp.* and *Verticillium sp.* Amongst the selected sites, maximum number of fungal species was found from Raisen Fort Raisen, Rajwada Palace Indore, Bhimbetka caves Raisen, Islamnagar Fort Bhopal and Bir Singh Palace Datia. The sites showing lesser fungal diversity as compared to these were Shaukat Mahal Bhopal, Sadar Manzil Bhopal, Siddhawat ghat Ujjain, Bharthari Caves Ujjain and Gohar Mahal Bhopal (Table 2, Fig. 6). The prevalence of fungal species from deteriorating sites are shown in Fig. 7.

Table 2: Distribution of positive samples in different sites

Site Code	Site Name	Number of samples examined	Number of positive samples	% Distribution of positive samples
RR	Raisen Fort Raisen	05	04	80
RI	Rajwada Palace Indore	07	06	85.71
BR	Bhimbetka caves Raisen	06	06	100
IB	Islamnagar Fort Bhopal	04	03	75
BD	Bir Singh Palace Datia	04	04	100
SB	Shaukat Mahal Bhopal	05	03	60
SMB	Sadar Manzil Bhopal	04	02	50
SU	Siddhawat ghat Ujjain	08	03	37.5
BU	Bharthari Caves Ujjain	08	07	87.5
GB	Gohar Mahal Bhopal	04	03	75
	TOTAL	**55**	**41**	**74.54**

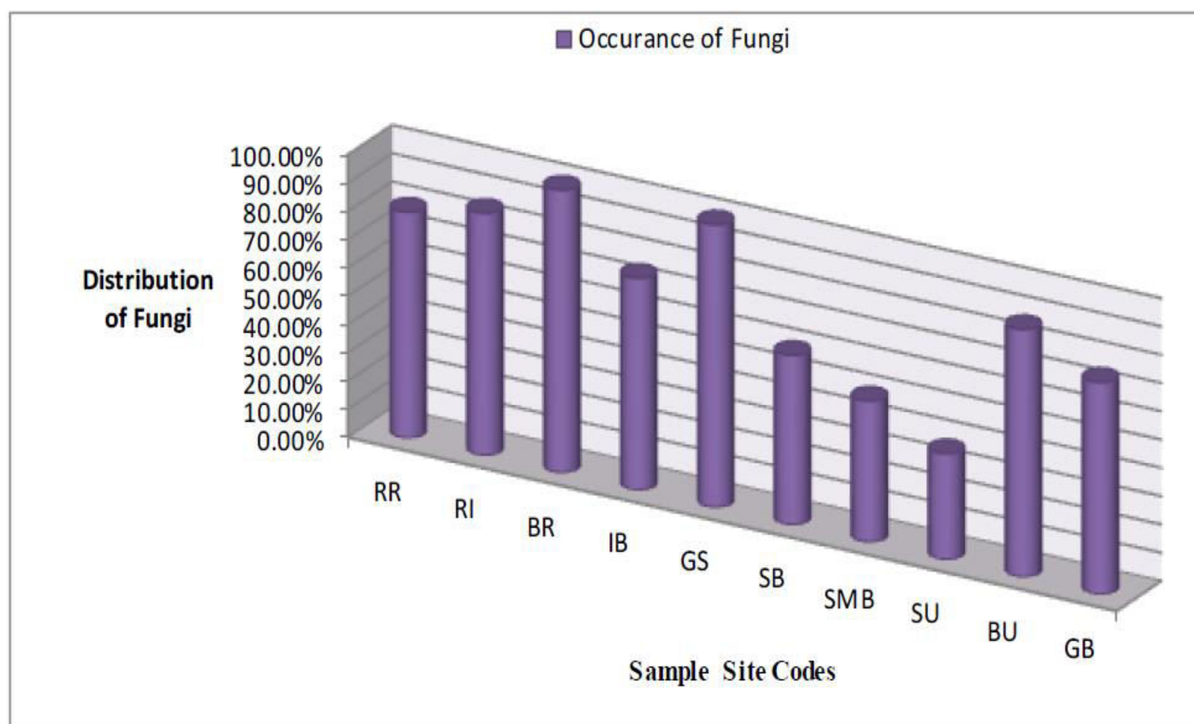

Fig. 6: Percent distribution of fungal isolates from deteriorating monuments

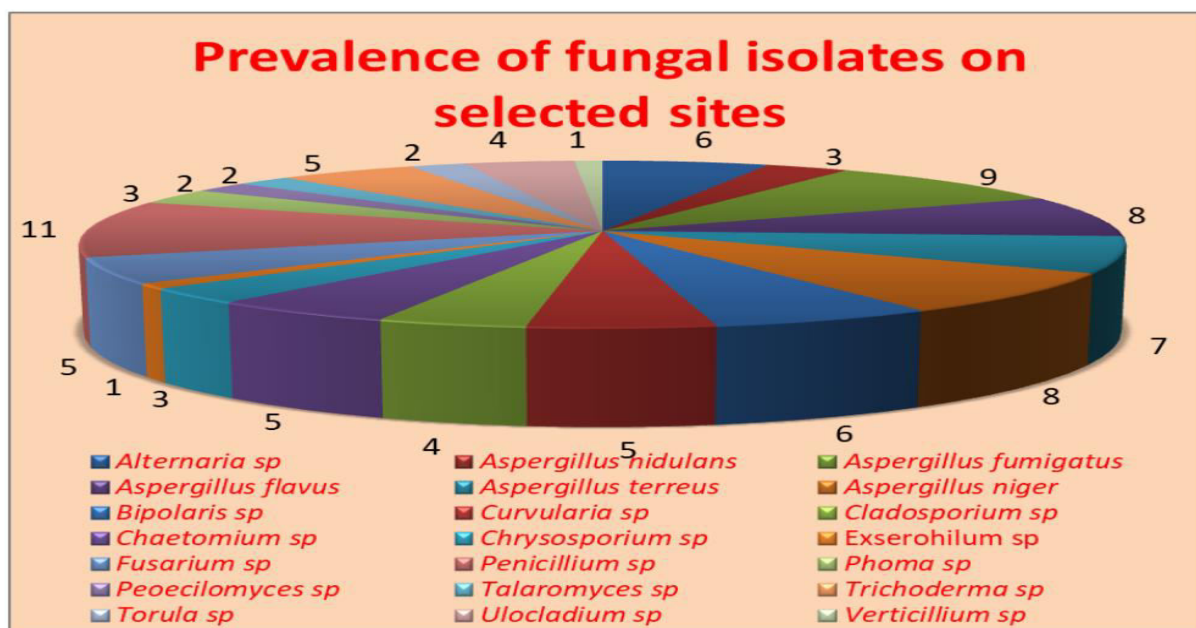

Fig. 7: Prevalence of fungal isolates from deteriorating monuments of Madhya Pradesh

4. DISCUSSION

The identified fungi found to be causing visible patina on the stone surfaces along with discolouration, exfoliation of stone material [27]. Some of the isolated fungi produce pigments and organic acids and are capable of hyphae penetration [28]. The production of organic acid was also seen by fungi *Penicillum frequentens* and *Cladosporium cladosporoides* which degraded the stone [29]. Previous studies have shown that large number of fungi show chemical decay properties [30]. Also fungal species *Cladosporium, Penicillum, Trichoderma, Fusarium* and *Phoma* are found more abundantly on the monuments, which is similar with our studies [31]. It was also reported in the earlier studies that *Aspergillus niger* is most abundant species found on limestone, marble and sandstone [32]. More amongst the previous studies *Curvularia* sp is common deteriorating agent associated with monuments [33]. *Aspergillus flavus* and *Fusarium roseum* was found on landsite, limestone, marble and sandstone [34]. There are many ways in which stonework on the monuments can be contaminated with salts [35]. Our findings correlate with the findings of other researchers [36].

5. CONCLUSION

The ancient monuments of Madhya Pradesh are under a constant threat of degradation by fungi. Due to the lack of maintenance, these historic buildings provide a rich source of organic residue over it, which favours microbial growth [37]. It can be concluded from this study that fungi plays a major role in the deterioration of these monuments. The data obtained during study will surely provide knowledge to select suitable strategy to eliminate fungal deteriogens and develop several restoration techniques [38]. The fungal diversity formulated from this study will help in generating new restoration approach [39]. Moreover it will help in implementation of strategies developed prior for these fungal species isolated from this study. By incorporating those preservation strategies, the monuments can be conserved for a longer period of time for future generations, thereby maintaining cultural heritages [40].

6. ACKNOWLEDGEMENTS

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Conflict of interest

The authors declare that there is no conflict of interest.

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