Fungal Species Associated with Deterioration of Selected Building Paints in Nile University of Nigeria, Abuja

Ezeagu G.G.*, Namla D., Saidu, M. and Muhammed S.S.D.
[1]Department of Biology, Microbiology & Biotechnology, Nile University of Nigeria, Abuja Nigeria.
gloria.ezeagu@nileuniversity.edu.ng; +234(0) 8060322809

Abstract
Fungal induced discolorations are deformative features occurring on building paints in tropical areas around the world. Paints on buildings are meant to protect surfaces from biodeterioration, corrosion, oxidation, environmental weathering or other types of deterioration. The actions/activities of biological deteriogens tend to defeat this aim. This study was carried out to identify fungal species associated with the biodeterioration of selected building paints at Nile University of Nigeria. A total number of twelve (12) samples were collected from areas of the buildings showing visible signs of discoloration. The suspected areas were gently rubbed with sterile medical rayon swabs, placed in dry sterile tubes and taken to the laboratory for further analysis. Following standard methods for the isolation and characterization of fungi and by using Sabouraud Dextrose Agar, Potato Dextrose Agar and peptone water as growth media, 9 fungal species were identified. The isolated fungi genera include Fusarium, Mucor, Aspergillus, Cladosporium, Rhizopus and Penicillium. Aspergillus species had 44.45% occurrence among all the fungal isolates and the high frequency of occurrence may be attributed to its high resistance to adverse environmental conditions. The study provides reliable data on the fungal communities implicated in the biodeterioration of building paints within the university.

Keywords: Biodeterioration, discolouration, *Aspergillus niger,* paints, deteriogens,

1 Introduction

Biodeterioration refers to any undesirable change in the properties of a material caused by the vital activities of organisms (Allsopp et al., 2010). It is the alteration of the quality, condition, appearance and/or function of a material or system by the growth and metabolic activities of micro and macro organisms. The breakdown activities of these living organisms reduce the material(s) to an inferior state, which makes it a matter of great concern, as this causes irreversible damages to building paints, textiles, plastics, pharmaceuticals and many cultural artifacts. The discoloration of building paints is a common issue mostly faced in tropical humid regions of the world. Although paints are meant to protect surfaces from biodeterioration, corrosion, oxidation, environmental weathering or other forms of deterioration, the action of biological deteriogens tend to defeat this aim (Ugbogu et al., 2017). The microbial deterioration of cultural heritage includes physical and chemical damages as well as aesthetic alteration (Cappitelli et al., 2020). Biodeterioration of wall paintings by microorganisms, known as biodeteriogens, including autotrophic and heterotrophic bacteria, microfungi, cyanobacteria, algae and lichens, represent an alarming challenge for the preservation of institutional, commercial and administrative buildings, as well as cultural heritages (Pinna, 2021). Studies have revealed that among the biodeteriogens, fungi are the most predominant group as they play an important role in the biodeterioration of building paints, due to the fact that the environment for their growth is more extensive. Garlarde & Morton, (2009), reported that the discoloration of building materials is often thought to be primarily due to fungi, since they can be highly pigmented. The fungi degrade the paint film by producing extracellular enzymes such as ligninase, cellulose and organic acids (Malathi & Devanathan, 2017).

The deteriogens biodeteriorate the paint constituents and reduces its economic value, durability, adhesive and decorative finish (Ugbogu et al., 2017). Precisely, variety of organic and inorganic materials present in paints such as sugars, gums, and other polysaccharides, proteins, linseed and other oils, waxes, etc, represents an optimal environment for biological colonization, which consequently causes aesthetic and structural damage of buildings (Caselli et al., 2018).

Very few studies have been carried out conventionally on the microbial community structure of discoloured painted walls in Nigeria, therefore, knowledge of the true microbial diversity is elusive (Obidi & Okekumjo, 2017). To ascertain and characterize the morphological features of the fungal biodeteriogens

adhering to the building paintings of Nile University of Nigeria buildings is crucial in developing new and precise painting mixtures with novel biocidal compounds against fungal growth. Also, a prompt strategy to limit further damage, evaluating and quantifying the presence of biological systems that induce damage in heritage materials is indispensable. This study therefore is primordial to determine the degree of association of fungal species with the building wall paintings in Nile University of Nigeria.

2 MATERIALS AND METHODS

Media Preparation

Two media, Potato dextrose agar (PDA) and Sabouraud dextrose agar (SDA) were prepared according to the manufacturer's instructions. 39g of PDA powder was dissolved in 1L of distilled water and 62g of SDA powder was dissolved in 1L of distilled water for PDA and SDA media. The media were sterilized and used to isolate fungi.

Collection of Samples

A total number of twelve (12) samples were collected from areas of the buildings showing visible signs of discolorations/biodeterioration using non-invasive sampling procedure, by gently rubbing sterile cotton paired swabs over 2cm2 of the areas visibly affected then stored in sterile tubes for transportation to the laboratory in accordance with Vacar et al., (2022). The present study was conducted during the 2022 dry season. Collected samples were taken to the Microbiology laboratory, Nile University of Nigeria, for further analysis. Sterile medical rayon swab was also rubbed on other part of the wall paints that showed no discolorations, and this was used as control.

Isolation of fungal colonies

The inoculum was prepared by adding 2ml of 0.1% peptone water into each sample in the sterile medical swab tubes, after which each tube was shaken and the swab used to spread the inoculum on the prepared sterile potato dextrose agar (PDA) and Sabouraud dextrose agar (SDA) plates (containing 50 µg/L of chloramphenicol) in duplicates. The culture plates were incubated at room temperature (28±2 ºC) for 7 days (Abdel-Kareem, 2010). The distinguishable colonies were subcultured on PDA plates and incubated at room temperature (30 °C) for 7 days to obtain pure isolates.

Identification of isolated fungi

The fungal isolates were morphologically identified using cultural and morphological features such as the growth pattern, conidial morphology, colony colour/pigmentation. The microscopic identification was carried out following standard laboratory techniques using Lactophenol cotton blue stain as described by Montanari et al. (2012). The identification was achieved by placing a drop of the stain on a clean slide with the aid of a mounting needle, a small portion of the aerial mycelia from the fungi cultures was removed and placed in a drop of lactophenol cotton blue stain. The mycelium was well spread on the slide with the needle. Carefully, the stain was covered with a clean sterile coverslip to avoid making air bubbles to the stain. The slide was then viewed under the light microscope at two magnification lenses, first at x10 lens and then at x40 for higher magnification. The microscopic features of the mycoflora were observed, recorded and identified in accordance with Samson &Varga, (2007) and www.universe84a.com(accessed on 18th May, 2023).

3 RESULTS

Cultural and microscopic characteristics of fungal isolates

A total of six (6) fungal genera comprising nine (9) species, which include Fusarium oxysporium, Mucor racemosus, Aspergillus flavus, Aspergillus niger, Aspergillus versicolor, Aspergillus sp., Cladosporium sp., Rhizopus sp., and Penicillium sp. were isolated in this study (Table 1).

The results in Table 1 showed the cultural features of the fungal species on the growth media. Isolate A0 showed white fluffy aerial mycelium while A1 was blackish and granular. B0 appeared with irregular gray pigmentation. C0, C1 and C2 had brownish-black, white to yellow and yellowish aerial mycelia respectively. SQ1 and SQ2 were black/blue and bluish while Kth isolate was yellowish green.

Microscopic examination showed septate hyphae for all fungal isolates except for B0 and SQ1, which showed aseptate hyphae. The kinds of asexual spores were conidia for A0, A1, C0, C1, C2, SQ2 and Kth isolates. B0 and SQ1 isolates however, had sporangiophores, bearing the spores (Table 1).

The genera, Aspergillus was predominant among the isolates with a 44.45% rate of occurrence while other genera each had 11.11% occurrence (Figure 1).

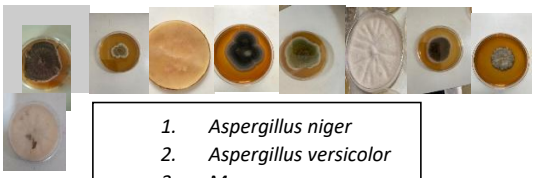
There were no observable fungal colonies on the control media plates after the period of incubation.

4 Discussion

This study was carried out on the walls of the buildings showing visible signs of discolouration/biodeterioration. The ability of the isolated fungal species to have impacted such discolouration on the walls of the buildings could be related to the reports of Ljaljević-Grbić & Vukojevic, (2009), who reported that these fungi must have induced mechanical exfoliation of building blocks and caused the colour changes.

Table 1. Colonies Morphology of isolates on Potatoes and Sabouraud Dextrose Agar

Isolate Code	Form	Elevation	Surface	Pigmentation	Structure of hyphae	Kind of asexual spore	Isolates
A_0	Circular	Columella	Fluffy	White	Septate	Round conidia	*Fusarium oxysporium*
A_1	Circular	Tapered	Granular	Blackish	Septate	Oval conidia	*Aspergillus niger*
B_0	Irregular	Columella	Effuse	Gray	Aseptate	Greenish spores	*Mucor racemosus*
C_0	Circular	Raised	Granular	Brownish black	Septate	Oval conidia	*Aspergillus* sp.
C_1	Irregular	Raised	Effuse	White/yellow	Septate	Oblong conidia	*Cladosporium* sp.
C_2	Circular	Curved	Effuse	Yellowish	Septate	Oval conidia	*Asp. versicolor*
SQ1	Circular	Tapered	Effuse	Black/blue	Aseptate	Sporangium	*Rhizopus* sp.
SQ2	Circular	Columella	Cottony	Bluish	Septate	Chains of conidia	*Penicillium* sp.
Kth	Irregular	Raised	Granular	Yellow-green	Septate	Yellowish conidia	*Aspergillus flavus*



1. *Aspergillus niger*
2. *Aspergillus versicolor*
3. *Mucor racemosus*
4. *Penicillium* species
5. *Aspergillus flavus*
6. *Cladosporium* species
7. *Aspergillus* species
8. *Fusarium* species
9. *Rhizopus* species

Note: NA denotes **Not Applicable.**

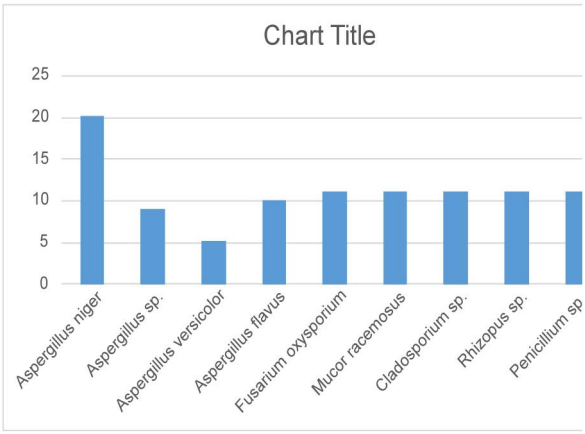


Figure 1: Fungal Genera Distribution and Percentage

The growth of the fungi on the building paints is also probably due to the favorable growth conditions such as the moisture content, availability of nutrients and temperature (Menega et al., 2016). The constituents of wall paintings including the pigment, binder, thinner and drier are all susceptible to microbial attack (Obidi & Okekunjo, 2017). The genera Aspergillus and Penicillium have been reported to adhere to favorable surfaces by releasing some organic acids, while Cladosporium species attach to surfaces via mechanical hyphae penetration and production of different pigments (Unkovic et al., 2018). Cladosporium was the most abundant fungal genus detected in the biofilm on the surfaces of all paint formulations at all sites after four years in a report by Shirakawa et al. (2010). That is however in contrast with the report of the present study, where four different Aspergillus species were isolated with Aspergillus niger with 20.05% frequency of occurrence was the most common among the identified fungi isolates (Fig. 1). Aspergilli are known as non-selective with respect to abiotic growth factors, as they can even grow at relatively low humidity and temperature range between 6 °C and 55 °C (Krijgsheld et al., 2013), hence they had highest frequency of occurrence in this study, which was conducted at the dry season of the year. The survival ability of these biodeteriogens with respect to resistance to desiccation and osmotic stress provides them with a clear advantage on the building paints, which dry constantly (Obidi & Okekunjo, 2017). As a result, they can occur as colloidal suspensions of fungal spores and conidia, increasing the concentration of pollutants and infectious agents in the dry air (D'Amato et al., 2018).

Aspergilli also secrete a series of enzymes that degrade polymers such as polyethylene, polystyrene and polyvinyl chloride. According to Krijgsheld et al. (2013), this group of fungi convert these polymers within their environment into molecules that serve as nutrients for their optimal growth, and cause the release of carbondioxide into the environment. Similarly, Horve et al. (2020), affirmed that the specialized biochemical functions and capabilities of these biodeteriogens give them the ability to survive under harsh climatic conditions, using the limited organic matter available on the paint films on building paints. The thick and melanized nature of the fungal cell wall is another survival mechanism used by fungi to resist the effect of chemical attack such as biocides present in the paint films (Obidi & Okekunjo, 2017). Fungal development on the superficial surface of painted area is a warning that when moisture is absorbed within the room walls and there is sufficient organic material on the walls to support fungal growth which can harm human health by inhaling thatspores (Bashir & Hafeez, 2016).

Aspergillus and Penicillium species dominate the biodeteriorative abilities usually screened in biodeterioration contexts (Trovao & Portugal, 2021). Ugbogu et al. (2017), reported that Aspergillus species recorded 66.67% occurrence while

Penicillium and Mucor each had 16.67% occurrence in their study. The above report and that of the present study are all in assertion that the genus Aspergillus are the predominantly isolated fungi from biodeteriorated painted walls.

Obidi and Okekunjo, (2017) reported that Fusarium and Aspergillus niger were among the fungi isolated from discoloured building walls, though at minimal population density. Shirakawa et al. (2010), in their research, reported that Mucor and Rhizopus are among the genera that are normal flora of painted surfaces. Rhizopus species was also isolated from biodeteriorated fabric paints by Malathi and Devanathan, (2017).

Microbiologically clean buildings probably do not exist, as some contamination begins as early as during the construction phase itself. Discoloration of paints on walls is usually an indication of presences of mould colonies, (Elumalai et al., 2014). Fungi require various favourable conditions for their growth in the buildings. Some of these conditions are favourable temperature (0- 25°C), nutrients, oxygen and water.

5 Conclusion

The isolated fungal species were involved in the discoloration of building wall paints in Nile University of Nigeria, Abuja. Aspergillus species, which had the highest frequency of occurrence may be having the highest resistance to adverse environmental conditions. Further studies during the rainy season is therefore recommended, with the assumption that rain could increase the abundance and diversity of the biodeteriogens. A prompt and effective conservation strategy to limit further damage on the buildings is recommended, and this may include among others, developing new and precise painting mixtures with novel biocidal compounds against fungal growths.

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