

## Engineering Case Report

# Evaluation of Five Antifungal Agents Used in Remediation Practices Against Six Common Indoor Fungal Species

### OVERVIEW

We investigated the effect of five antifungal agents (Sanimaster, hydrogen peroxide, isopropyl alcohol, bleach, and Sporicidin) used in fungal remediation practices on the growth and spore germination of six commonly occurring indoor fungal species (*Alternaria alternata*, *Aspergillus niger*, *Chaetomium globosum*, *Cladosporium herbarum*, *Penicillium chrysogenum*, and *Stachybotrys chartarum*). These antifungal agents significantly inhibited the growth and spore germination within 12 hr of treatment. When the antifungal agents were washed off with distilled water, no significant differences were observed in spore germination after 24 hr of incubation period. Two weeks after treatment, in vitro fungal growth was not inhibited compared with non-treated control. In the treated wood blocks, colony forming units of these fungi were viable after 2 weeks of treatment.

### INTRODUCTION

Society has been concerned with indoor air quality (IAQ) since biblical times.<sup>(1)</sup> During the 1960s, IAQ became an important issue when researchers found that indoor pollutant levels in many cases exceeded those of outdoor levels.<sup>(2)</sup> Studies have shown that most of us spend 90% of our time indoors inhaling approximately 15 m<sup>3</sup> of ambient air each day.<sup>(3)</sup> Therefore, IAQ is essential and critical to our health.

Recently, fungi (both mold and yeast) have become one of the leading causes of IAQ complaints.<sup>(4–11)</sup> Fungi become a problem within a built environment where excessive humidity or moisture is present for an extended period of time.<sup>(5–19)</sup> The problem can originate from sudden water releases such as a ruptured pipe or large spill that goes untreated, or from a chronic condition such as a leaking roof or plumbing failure. Even high humidity or warm, moist air condensing on cool surfaces can cause fungal problems. Studies have shown that fungal occurrence in indoor environments was high during fall and summer seasons.<sup>(20)</sup> Environmental factors such as high temperature and relative humidity are among the factors contributing to the higher occurrence of indoor fungi.

Fungi can grow almost anywhere in a building if conditions are favorable (moisture, temperature, and substrate) for their growth and activities.<sup>(21–23)</sup> If there is visible fungal growth on painted wall surfaces, it is possible that fungi may also be growing inside the wall cavity. The environment inside the walls of a structure often differs drastically from the outside and could create perfect conditions for fungal growth. If the wall remains wet for a prolonged period, fungal growth on the back side of gypsum board will likely be worse than that on the front. A noninvasive but limited approach

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for observing fungal growth within the interstitial space (e.g., wall or ceiling cavity) can be performed using a borescope. Air sampling for the presence of fungal spores within the interstitial space can be performed using an inner wall sampling adapter with sampling cassette connected to an IAQ sampling pump.

Fungal contamination in the indoor environment is a complex issue and can cause health hazards for the inhabitants. All fungi are potentially harmful when they are allowed to grow in the indoor environment. Whether dormant, viable, or non-viable, fungal spores can be harmful when inhaled. Fungal contamination of the indoor environment has been linked to health problems including headache, allergy, asthma, irritant effects, respiratory problems, mycoses (fungal diseases), and several other non-specific health problems.<sup>(24)</sup> The longer fungi are allowed to grow indoors, the greater the chances are that fungal spores may become airborne and cause adverse health effects. If indoor fungal contamination is not effectively remediated, fungal problems can spread to other non-affected areas and can cause health problems to the occupants. Inhalation of fungal spores is implicated as a contributing factor for organic dust toxic syndrome and non-infectious fungal indoor environmental syndrome.<sup>(25)</sup> In addition, concentrations of mycotoxins found in buildings have damaged cells of the central nervous system.<sup>(26–28)</sup>

For this study, five commonly used antifungal products to remediate fungal contamination were identified through interviews with independent remediation contractors and franchise owners of national remediation companies representing ServPro, ServiceMaster, and The Serum System. Some products fail to kill fungal spores; others make fungal spores dormant but fail to prevent the future fungal growth. Still other products kill fungal spores and prevent the future mold growth but are themselves toxic to homes and their inhabitants. Fungal remediation refers to the process of removing contamination together with steps to modify the indoor environment to prevent the future fungal growth.

The objective of this study was to evaluate the efficacy of five antifungal agents commonly used in remediation practices to eradicate and prevent future fungal growth.

## METHODS

### Antifungal Agents and Fungal Species

Antifungal agents used in this study are Sanimaster (contains 5–20% quaternary ammonium chloride compounds, 1–5% ethanol, 1–5% non-ionic surfactant, and 1–5% chelating agent); hydrogen peroxide (17%); isopropyl alcohol (70%); bleach (contains 6.15% sodium hypochlorite and <1% sodium hydroxide); and Sporicidin (contains total phenol 1.93% and glutaraldehyde 1.12%).

Six commonly occurring fungal species found in the indoor environment<sup>(10,29–31)</sup> were used in this study. These were *Alternaria alternata* (Fr.) Keissl., *Aspergillus niger* van Tieghem, *Chaetomium globosum* Kunze ex Steud., *Cladosporium*

*herbarum* (Pers.) Link ex Gray, *Penicillium chrysogenum* Thom., and *Stachybotrys chartarum* (Ehrenb. ex Link) Hughes.

### Preparation of Spore Suspensions

Spore suspensions were prepared by addition of 9 ml of peptone physiological salt solution (8.5 gm of NaCl liter<sup>-1</sup> with 1 gm of bacteriological peptone (Oxoid) liter<sup>-1</sup>, supplemented with 0.1% Tween 80) to the culture. Suspensions were prepared from 25 tubes and filtered through a 17- $\mu$ m-pore-size nylon filter, reaching a final volume of 180 ml. Subsequently, the suspensions were centrifuged (4000 g) for 3 min. Spores were resuspended in 2 ml of malt extract broth medium (CM57; Oxoid), and the suspension was adjusted to pH 4.0 with lactic acid. The spore suspensions were adjusted to densities of 10<sup>8</sup>, 10<sup>7</sup>, 10<sup>6</sup>, and 10<sup>5</sup> spores ml<sup>-1</sup>, and cells were counted in a hemocytometer.

### Effect of Five Antifungal Agents on Spore Germination

*Alternaria alternata*, *A. niger*, *C. globosum*, and *C. herbarum* were grown on 2% malt extract agar (MEA) at 25°C in the dark for 1 week, whereas *S. chartarum* was grown on cellulose agar (CA) for 2 weeks. The spore suspension was prepared by transferring from the fungal culture with a transfer loop into 9 ml sterile distilled water, and the concentration of the suspension was adjusted to approximately 10<sup>5</sup> spores/ml.

To test the effect of five antifungal agents (Sanimaster, 17% hydrogen peroxide, 70% isopropyl alcohol, bleach, and Sporicidin) on the spore germination of six fungi, 10  $\mu$ l of spore suspension was mixed with 10  $\mu$ l of filter sterilized antifungal agents mentioned above in a cavity slide. Slides with spores were kept moist by placing them on glass rods on the moistened filter paper in petri dishes and sealed with Parafilm. Spore germination was recorded after 12 hr incubation at 25°C in the dark, and 100 spores were counted in each of four replicates.

### Sensitivity of Spores to Five Antifungal Agents

Non-germinated spores exposed to antifungal agents were washed with sterile distilled water and transferred to freshly prepared plates of water agar without antifungal agents. These were incubated as described above and the percentage of spore germination was recorded after 24 hr.

### Effect of Antifungal Agents on Radial Growth of Fungi

For this experiment, 90-mm plastic petri plates containing MEA (for *A. alternata*, *A. niger*, *C. globosum*, and *C. herbarum* and CA (for *S. chartarum*) media and five antifungal agents described above were used. One ml of the filter sterilized antifungal agents was added separately to the petri plates. There were five replicates for each antifungal agent. Following treatments resulted:

Sanimaster + *A. alternata*, hydrogen peroxide + *A. alternata*, 70% isopropyl alcohol + *A. alternata*, bleach + *A. alternata*, and sporicidin + *A. alternata*.

Sanimaster + *A. niger*, hydrogen peroxide + *A. niger*, 70% isopropyl alcohol + *A. niger*, bleach + *A. niger*, and sporicidin + *A. niger*.

Sanimaster + *C. globosum*, hydrogen peroxide + *C. globosum*, 70% isopropyl alcohol + *C. globosum*, bleach + *C. globosum*, and sporicidin + *C. globosum*.

Sanimaster + *C. herbarum*, hydrogen peroxide + *C. herbarum*, 70% isopropyl alcohol + *C. herbarum* bleach + *C. herbarum*, and sporicidin + *C. herbarum*.

Sanimaster + *S. chartarum*, hydrogen peroxide + *S. chartarum*, 70% isopropyl alcohol + *S. chartarum* bleach + *S. chartarum*, and sporicidin + *S. chartarum*.

Control plates contained abovementioned fungal species and sterile distilled water was added instead of antifungal compounds.

The plates were incubated at 25°C in the dark for 2 weeks. The diameters of the colonies on the bottom layer were measured using a ruler.

### **In vitro Treatment of Antifungal Agents on Inoculated Woods**

Small blocks (16 × 8 cm) of pine wood free from decay and stain were cut from 2 × 4 pine wood boards (commercially purchased). All the blocks were autoclaved for 2 hr at 121°C. When cooled, blocks were separately inoculated with 5 ml spore suspension (10<sup>5</sup> spores/ml) of *A. alternata*, *A. niger*, *C. globosum*, *C. herbarum*, *P. chrysogenum*, and *S. chartarum*. The control blocks received 5 ml of sterile distilled water. There were three replicates for each fungal treatment. The blocks were then placed in a plastic zippered bag, kept moist, and incubated at 25°C for 8 weeks. The blocks were examined periodically to observe the fungal growth. After 8 weeks when the considerable fungal growth was observed, the wood blocks were separately treated with five antifungal agents Sanimaster, 17% hydrogen peroxide, 70% isopropyl alcohol, bleach, and Sporicidin. After 1 hr, the fungal growth was removed from the blocks. The blocks were allowed to air dry. For hydrogen peroxide, wood blocks were treated twice (6 hr of intervals) and fungal colonies were removed by brushing (as recommended by the remediation company).

### **Effect of Antifungal Treatment on Fungal Growth on Remediated Wood Blocks**

When fungal growth was cleaned with antifungal agents and growth was removed (as recommended by remediation companies), wood blocks were allowed to dry in ambient air for 5 weeks. After the blocks had dried, swab samples were taken from both treated and non-treated wood blocks. Swab samples were then aseptically inserted into culture tubes containing 10 ml of sterile distilled water. Culture tubes were vortexed for 10 sec, and 1 ml of the solution was poured into MEA medium for *A. alternata*, *A. niger*, *C. globosum*, and *C. herbarum*, and CA medium for *S. chartarum* in the petri plates for individual antifungal agent and fungal species. The plates were incubated at 25°C for 1 week with *A. niger* and

*P. chrysogenum* (fast-growing fungi), and with *A. alternata*, *C. herbarum*, *C. globosum*, and *S. chartarum* (slow-growing fungi) for 2 weeks in the dark. Colony forming units (CFU) of each fungal species were recorded when fungal growth was observed.

All the experiments were repeated three times. Data presented were means from one of the experiments, with three experiments showing similar results.

### **Statistical Analysis**

Data were subjected to analysis of variance.<sup>(32)</sup> The individual means were compared using the Scheffe's test for multiple comparisons using SAS software, version 9.0.<sup>(33)</sup> Means followed by the same letters (a,b,c, and so on) in bars (in the graphs) for a particular fungal species against antifungal agents are not significantly ( $P = 0.05$ ) different from each other by Scheffe's test for multiple comparison.

## **RESULTS**

### **Effect of Five Antifungal Agents on Spore Germination**

The spore germination of *A. alternata*, *A. niger*, *C. globosum*, *C. herbarum*, *P. chrysogenum*, and *S. chartarum* was significantly inhibited when treated with Sanimaster, hydrogen peroxide, isopropyl alcohol, bleach, and Sporicidin after 12 hr of treatment (Figure 1).

### **Sensitivity of Five Antifungal Agents on Spore Germination**

These antifungal agents significantly inhibited the growth and spore germination within 24 hr of treatment. However, when the antifungal agents were washed off with distilled water, no significance differences were observed in spore germination after 24 hr of incubation period (Figure 2).

### **Effect of Five Antifungal Agents on Radial Growth**

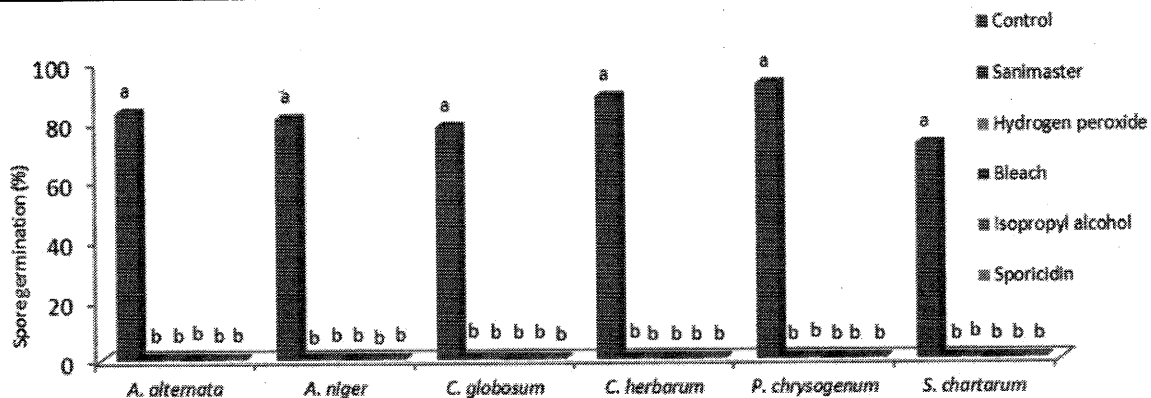
No significant differences in radial growth of the fungi were recorded between treated with antifungal agents and non-treated control plates after 2 weeks of incubation period (Figure 3).

### **Effect of Five Antifungal Agents on Colony Forming Units**

Fungal growth was observed when swab samples were taken both from the treated and non-treated control wood blocks 5 weeks after treated with antifungal agents (Figure 4).

## **DISCUSSION**

Our results show that fungal growth including spore germination and colony forming units were significantly inhibited one week after treatment. However, when treated spores were washed with distilled water and antifungal compounds



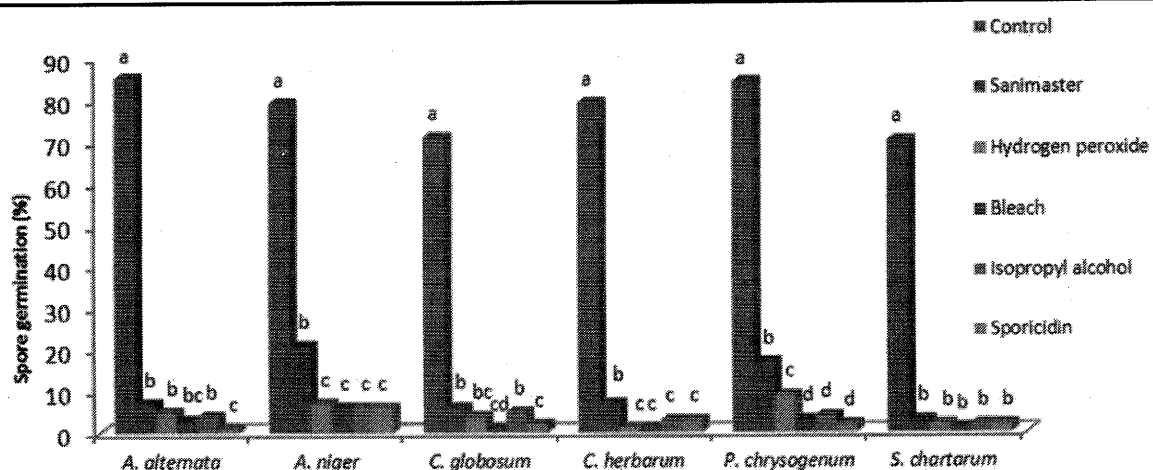
**FIGURE 1.** Effect of five antifungal agents on spore germination of six fungal species after 12 hr of incubation. Values are the means of 20 replicates. Means followed by the same letters (a,b,c, and so on) in bars for a particular fungal species against antifungal agents are not significantly ( $P = 0.05$ ) different from each other by Scheffe's test for multiple comparison.

were removed, these fungi recovered from the initial shock and spores became viable. All the antifungal compounds' treatments showed similar results. The toxic effects of these chemicals were reduced and most of these fungi become viable after a period of time. On the antifungal-treated wood blocks and after removing the fungal growth, small amounts of fungal inoculum were present and they remained in a dormant stage. When swab samples were taken from these treated wood blocks and inoculated onto suitable nutrient media, fungi came out of dormancy and became viable as indicated by their CFU onto the media.

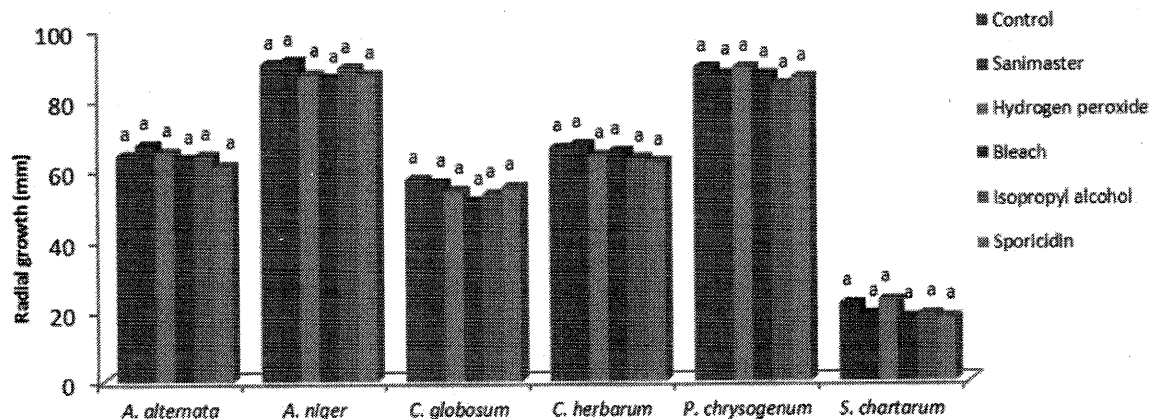
Our results show that all the tested fungi showed effect of fungistasis or mycostasis, a phenomenon linked to exogenous dormancy where fungal growth is inhibited without any effect on viability.<sup>(34-36)</sup> This inhibition is due to inhibitory effect

of antifungal compounds when applied on the fungal contaminated surfaces. The inhibitory effect is reversible once the inhibitory substances are removed or become diluted; spores again become viable and mycelia can resume growth. Most of the fungicides are effective only on hard non-porous surfaces. Viable spores hiding in porous surfaces may be unaffected and can go dormant when fungicides are applied. The peak and valley terrain of porous substrates provide easy cover for micron-size spores eluding direct contact with viscid antimicrobial agents.

Correcting fungal contaminants requires understanding the extent of the problem and the underlying causes. In many cases, this is quite simple, for example when an obvious moisture source has affected only a small area resulting in observable visible fungal growth. However, this can be difficult



**FIGURE 2.** Sensitivity of five antifungal agents on spore germination of six fungal species after 24 hr of incubation. Values are the means of 20 replicates. Means followed by the same letters (a,b,c, and so on) in bars for a particular fungal species against antifungal agents are not significantly ( $P = 0.05$ ) different from each other by Scheffe's test for multiple comparison.



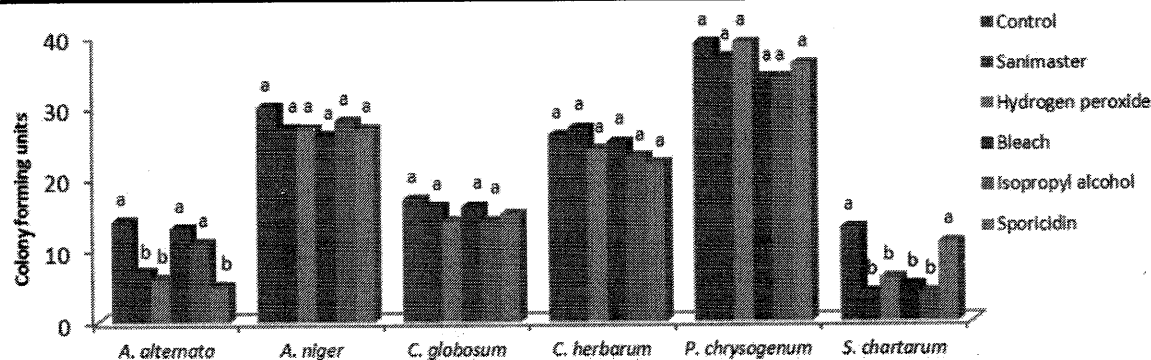
**FIGURE 3.** Effect of five antifungal agents on in vitro growth of six fungal species after 2 weeks of incubation in the petri plate. Values are the means of 20 replicates. Means followed by the same letters (a,b,c, and so on) in bars for a particular fungal species against antifungal agents are not significantly ( $P = 0.05$ ) different from each other by Scheffe's test for multiple comparison.

when the source(s) of moisture, their interaction with building conditions, or the location(s) of the growth are not readily apparent. When a complex fungal problem exists, it is wise to carefully assess the problem thoroughly and objectively before the beginning of remediation. To achieve a durable and effective solution, it is also imperative to understand the reason(s) for the moisture problem(s). Once pathways of moisture are known, then it becomes easy to locate hidden fungal growth. Knowing the source of the excess moisture is vital to correct it and prevent recurrence of the problem.

The success of remediating a large-scale fungal problem ultimately depends on how well the moisture and contamination problem is understood. If planning the remediation relies heavily on reports from past investigations, the accuracy and completeness of those efforts should be objectively assessed. It is essential to review the findings in the

reports and evaluate how completely the important issues were assessed.

It is very important that fungal growth be physically removed and contained to prevent cross contamination of the living space. Attempts to kill or inactivate fungal growth and spores with products such as fungicides, heat, or fogging does not eliminate spores. The remediated area should be properly cleaned and dried. Even a small number of dormant fungal spores can grow vigorously when conditions become favorable for their growth and activities. Inhaling large number of dead or dormant fungal spores can be harmful and cause health hazards to building occupants. Also, the chemicals used to treat or limit fungal growth can be very harmful. If biocides are used they must be registered with the state government pesticide control board and must be in accordance with state or federal government laws by a licensed pesticide applicator or licensed remediation companies.



**FIGURE 4.** Effect of five antifungal agents on colony forming units of six fungal species 5 weeks after remediated on wood blocks. Values are the means of 20 replicates. Means followed by the same letters (a,b,c, and so on) in bars for a particular fungal species against antifungal agents are not significantly ( $P = 0.05$ ) different from each other by Scheffe's test for multiple comparison.

## CONCLUSION

Our finding indicates that the commonly used fungicides in the indoor environment cannot completely kill all the fungal inocula. Most of the fungi form dormant spores when exposed with fungicides. These dormant spores can germinate and resume growth when a favorable environment is available to them. The results provide further evidence that physical removal of indoor fungal contaminated material is necessary as a proper remediation practice when dealing with indoor air quality problems. Our study strengthens the evidence that effect of fungistasis or mycostasis, a phenomenon linked to exogenous dormancy where fungal growth is inhibited without any effect on viability.

## DISCLAIMER

The information presented in this article is solely for the information of the reader. Authors are neither recommending nor rejecting the use of these antifungal agents in remediation practices.

## ACKNOWLEDGMENTS

We would like to thank The Serum System, Service Master, and ServPro for providing us with antifungal agents used in this study. We also thank Mike Kovar, Bryan Kovar, and Chris Kovar for their technical assistance and valuable advice.

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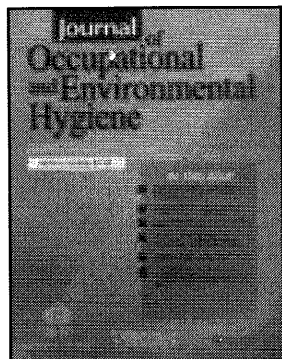
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## Journal of Occupational and Environmental Hygiene

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/uoeh20>

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Accepted author version posted online: 23 Oct 2012. Version of record first published: 29 Nov 2012.

**To cite this article:** P. Chakravarty & Brad Kovar (2013): Evaluation of Five Antifungal Agents Used in Remediation Practices Against Six Common Indoor Fungal Species, Journal of Occupational and Environmental Hygiene, 10:1, D11-D16

**To link to this article:** <http://dx.doi.org/10.1080/15459624.2012.740987>

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