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Original Research Article

A Survey of the Spread of *Stachybotrys chartarum* to Different Places in Baghdad

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Abstract: The study was conducted to investigate the contamination of buildings and bathrooms with the fungus (*Stachybotrys chartarum*). 50 samples were taken from low-ventilation bathrooms that showed signs of contamination at the sample site, which was divided into public bathroom, school, hospital, old home, and modern home. 35 were isolated. An isolate of the *S. chartarum* fungus, with 10 isolates per site. The isolation rate from public bathrooms and old house was 100%, while it was 70% and 80% for school and hospital, respectively. While the isolates were 0 from samples taken from modern house, the isolates were subjected to test the phenotypic diagnosis, such as the variation in colony color and colony density, as well as the microscopic diagnosis, which included the color of the fungus under the microscope, the shape of the conidia, and the distribution of the conidia.

Keywords: *Stachybotrys chartarum*, bathrooms, colony, conidia.

INTRODUCTION

The greenish-black fungus *Stachybotrys chartarum* belongs to the phylum Deuteromycota, which is made up of all fungal species whose sexual reproduction stage is currently unknown. Spore produced by conidia are asexual (Samson *et al.*, 2010). The species can be distinguished from other molds in indoor air that may contaminate materials in buildings that have seen water leaks by the shape and color of conidia and other structures that are inspected under a microscope. In wet cellulosic materials like drywall (wallboard, gypsum boards, sheetrock, wood, and wallpaper rich in cellulose), *S.chartarum* may eventually outcompete other molds that also produced colonies (Lombard *et al.*, 2016). The general public, whose homes and workplaces may be contaminated after a water spill, as well as workers involved in mold restoration, should be concerned about exposure to *S. chartarum* due to its potential to produce toxic macrocyclic trichothecenes and hemolytic stachylysins, which may be linked to idiopathic pulmonary hemorrhage in infants. Particles such as conidia, dry hyphae, and other fragments can be inhaled through mechanical aerosolization (Gnat *et al.*, 2021).

Slow-growing *S. chartarum* does not entirely outcompete other fungi in its niche market. This mold is uncommon in nature and seldom grows in the kind of expansive habitat that humans occasionally create (i.e., significant temperature swings, a lot of cellulose, no other molds, no sunlight, low nitrogen, and plenty of steady humidity). Spores are only discharged into the surrounding atmosphere when the fungus are physically moved, especially when they are damp. It is thought to be an uncommon indoor air pollutant (Donald and Barceloux March 2012), (Doherty *et al.*, 2011).

S. chartarum is a filamentous and cellulose-degrading fungus that is widely distributed throughout the world. As with most fungi, temperature, humidity, relative humidity, and growth substrate are all important factors that affect their growth. *Stachybotrys* species can grow in a wide range of temperatures, and requires a moisture content of at least 20% in the substrate, a relative humidity of 75% to 85%, and a substrate with a

Cellulose-based materials, high cellulose content, including hay, straw, plant residues, Grains and a variety of wet, temperature-variable building materials, like fiberboard and gypsum lining paper, offer the perfect environment for the mold to thrive (Guo., 2011; Al-Shammari *et al*., 2023). Various indoor locations such as carpets, furniture, bathrooms, wallpaper, and potted plants may serve as amplification sites for fungal development. Fungal spores can also be released into indoor environments through open windows and natural doorways. Mechanical ventilation systems (Asadi *et al*., 2011).

Stachybotrys is usually found in air samples from living environments. Many fungi can produce a large number of secondary metabolites, and some secondary metabolites pose a potential risk to human health. These toxic metabolites are called mycotoxins. (Sun *et al*, 2020) Since the 1930s of the previous century, reports of health issues in humans and animals associated with this fungus have been made. S. chartarum has lately been connected to the phenomenon known as "ill building syndrome." In the scientific literature, the association hasn't been consistently demonstrated, though (Miller and McMullin, 2014).

The US Centers for Disease Control confirmed in 1994 that exposure to abnormally high concentrations of *S. chartarum* spores caused pulmonary hemosiderosis, or bleeding in the lungs, in some infants in Cleveland, Ohio, leading to illness and even death (Wolf and Lai 2020).

S. chartarum contains two chemotypes, Atranone is the first mycotoxin produces and the other is mycotoxins produces are Trichothecene like satratoxin H (Ulrich *et al*., 2020). It is reasonable to assume that severe and prolonged exposure to inhaled dust particles, spores, and fungal fragments containing trichothecene may result in the development of human disease associated with toxin exposure. Conidia of *Stachybotris* species have been shown to contain trichothecene mycotoxins (Yang *et al*., 2019).

METHODS

Samples collection

In this study, Fifty samples of *S. chartarum* were collected from different resources: public bathroom, school, hospital, old home, and modern home. the samples taken by swabs from the places of contamination and placed in a container tube on distilled water and transferred to the laboratory for cultured and diagnosis.

Figure 1: *Stachybotrys chartarum* in public bathroom

Figure 2: *Stachybotrys chartarum* in old home

Figure 3: *Stachybotrys chartarum* in hospital

Figure 4: *Stachybotrys chartarum* in school

Isolation and Diagnosis

Using the streak plate method, the samples in tubes were initially spread on Petri dishes with Potato Dextrose Agar (PDP) supplemented with 250 mg/L of chloramphenicol. then incubated for seven days at 27 °C±2. Each fungal colony's brief mycelium was aseptically transferred to a new plate containing the medium. Sub-culturing the fungi repeatedly until pure fungi were isolated served as a purification method. A tiny portion of each isolate was taken using a sterile inoculating needle, mixed in the center of a clean microscopic slide with a drop of Lactophenol cotton blue stain, covered with cover slips, and examined under a microscope. The identification was done using common textbooks like (Barnett and Hunter, 1998; Andersen *et al*., 2003).

RESULTS AND DISCUSSION

Isolation and Diagnosis

The results showed the isolation of (35) isolates of the *Stachybotrys chartarum* fungus, divided around four places where contamination was concentrated, where the highest number of isolates were 10 isolates out of 10 samples in each of the public restrooms and the old house, i.e. a percentage of 100%, while the isolates were 7 in the school, i.e. a percentage. 70%, and the isolates taken from the hospital were 8, meaning 80%. As for the ten samples taken from the modern home, all of them tested negative, as shown in Table 1.

These results are consistent with many studies and research on the isolation of the *Stachybotrys chartarum* fungus from different places, and the comparison made in this research between the old house and the modern house gives us a future look at ways to combat black mold, use controlled building materials, and add some anti-fungal materials (Reynolds *et al*., 2012).

In the modern house, the relative humidity of the bathrooms was measured, as it did not exceed 40%, which means that the relative humidity limited the growth of Stachybotrys chartarum in the modern house, unlike the old house, in which the relative humidity reached 90%, and the school and hospital had a humidity of 85% (Menneer *et al*., 2022) (Buerman *et al*., 2019).

The Phenotypic diagnosis was based on the color of the fungal colony that appeared on the PDA dish as well as the size and density of the colonies, While the microscopic diagnosis of fungi was dependent on the length of the conidiaphore and the shape of conidia of the main (Betancourt *et al*., 2006).

Table 1: Distribution of *Stachybotrys chartarum* according to isolation location

Place	Number of sample	Number of isolates	Percentage of isolates
public bathrooms	10	10	100%
old houses	10	10	100%
school	10	7	70%
hospital	10	8	80%
modern house	10	0	0%

Figure 5: *Stachybotrys chartarum* under microscope (40X)

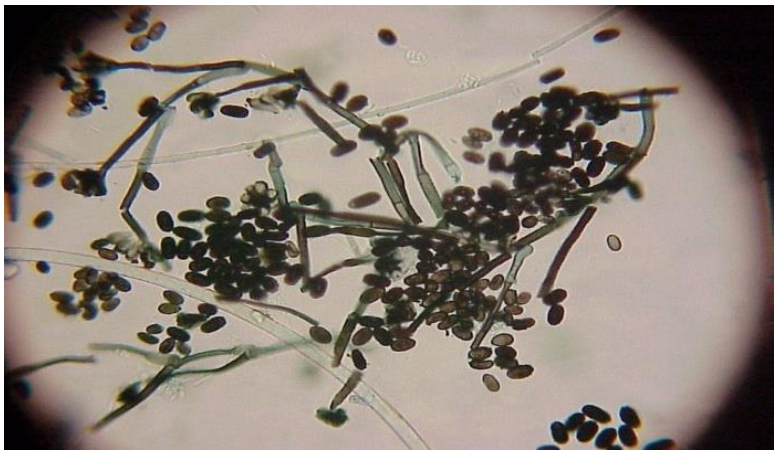

Figure 6: *Stachybotrys chartarum* under microscope (40X)

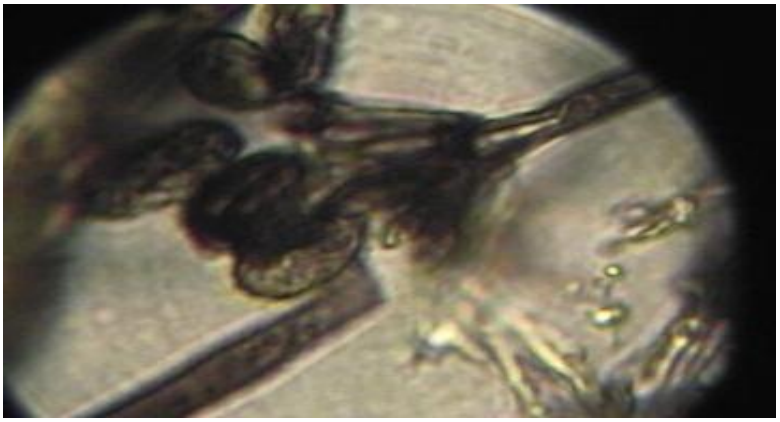

Figure 7: *Stachybotrys chartarum* under light microscope (near view)

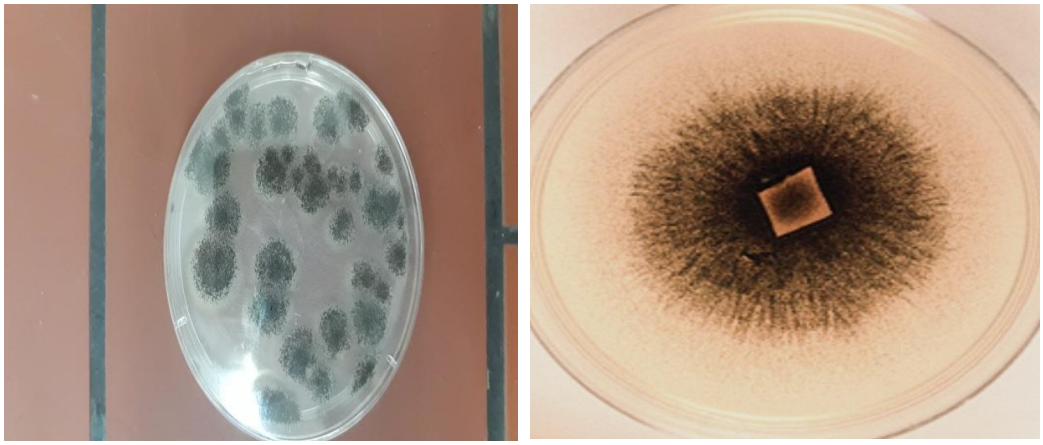

Figure 8: Single isolated colony of fungi in PDA plate

CONCLUSIONS

Indoor settings are typically contaminated with fungi, and the development of mould in buildings can result in many health problems, including skin rashes, headaches, dizziness, and other ailments, because this fungi can grow by spreading spores through the air.

Advises for Preventing Fungi

- ❖ Keep humidity as low as possible, less than 50% - throughout the day, assisted by an air conditioner or humidifier.
- ❖ Ventilate the house well, use hoods in the bathroom and kitchen, to pull the air out of the house.
- ❖ Fix any leaks on the ceiling, walls or plumbing, so that the mold does not find a suitable medium to grow.
- ❖ Clean bathrooms with deadly mold products.

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