Colorado State University

Environmental Health Services
Fort Collins, Colorado 80523-6021
(970) 491-6745
FAX: (970) 491-4804
http://chemdat1.ehs.colostate.edu

LABORATORY REPORT

Background

Stachybotrys chartarum Corda (1837) has emerged as an economically important fungal contaminant in water damaged buildings. The organism is capable of producing a variety of mycotoxins that demonstrate profound neural and hemorrhagic effects in humans. Because of these toxins, current remediation practices mandate the complete removal of all source material. The economic impact of *Stachybotrys* remediation and other indoor fungi is expected to exceed $100 million this year in the United State alone.

In support of their Department of Energy contracts, Sandia National Laboratories (SNL) developed a compound designed to decontaminate chemical and biological warfare agents. A license agreement was awarded to Modec, Inc. to manufacture and sell the SNL formula offered as MDF. This compound has been widely tested on mustard gas and Anthrax spores, but its efficacy against fungi was unknown. The following study at Colorado State University was designed to test the effectiveness of the MDF in killing *Stachybotrys chartarum*. Preliminary studies were also completed with the University of Maryland on the ability of the compound to neutralize the major *Stachybotrys* toxins.

Preliminary results indicate that a 10% dilution of the MDF will kill 99.99999% (>6.89 $\log_{10}$) of *Stachybotrys chartarum* spores under laboratory conditions. The formula was also found to neutralize *Stachybotrys* toxins when the organism was grown on rice and wet drywall.

Methods

Disinfectant Formulation

Equal parts (by volume) of the MDF were mixed with 7.99% Hydrogen Peroxide immediately prior to mixing with the test organisms or test substrates.

Test Organism

Stachybotrys chartarum laboratory strain 1299
Isolated from saturated drywall at Colorado State University

Stock Suspension

After positive microscopic identification on the drywall sample, the organism was struck onto five plates of Malt Extract Agar (MEA). The plates were incubated at 25°C for seven days in the dark. The MEA plates were flooded with 10 mL of sterile phosphate buffer each. Spores were dislodged from the surface of the plates by rubbing with a sterile pipette. The spore suspensions were combined into four centrifuge tubes. The spore suspensions were spun at 15,000 rpm for 15 minutes. The supernate was discarded and the pellets were rehydrated with phosphate buffer and combined into a single centrifuge tube. This wash step was repeated once again, finally suspending the pellet in 100 mL of sterile phosphate buffer. The stock suspension was refrigerated for storage.

Fungicidal Efficacy

The stock suspension was serially diluted and plated on MEA to determine the original spore count. A 5.0 mL aliquot of the stock suspension was then transferred to 10 sterile test tubes (5 concentrations in duplicate). Various dilutions of the MDF were then prepared by diluting the original concentrate with sterile distilled water. A 5.0 mL aliquot of the appropriate dilution was then added to each tube containing Stachybotrys spores. The disinfection reaction was allowed to continue for two minutes and then the spore/disinfectant solutions were immediately diluted and plated on MEA. The plates were incubated at 25°C for seven days and counted. The following dilution combinations were used:

- 5 mL spores with 5 mL 100% MDF = 50% SNL
- 5 mL spores with 5 mL 20% MDF = 10% SNL
- 5 mL spores with 5 mL 2% MDF = 1% SNL
- 5 mL spores with 5 mL 0.2% MDF = 0.1% SNL
- 5 ml spores with 5 mL 0.02% MDF = 0.01% SNL.

Toxin Neutralization

In the first toxin study, 1.0 mL of the stock suspension was spread onto each of 8 MEA plates and 4-3 inch diameter circles of drywall saturated with sterile water. The plates and drywall samples were incubated at 25°C for 10 days to allow for adequate toxin production. Four of the MEA plates and two of the drywall circles were flooded with 30 mL of freshly prepared 100% MDF formula. The disinfection reaction was allowed to continue for 10 minutes and then the surfaces were flooded with 100 mL of sterile water. The mycotoxins were extracted with 95% ethanol and centrifuged at 15,000rpm for 10 minutes. The supernate was transferred to a clean tube and sealed. The tubes were sent to Dr. Bruce Jarvis at the University of Maryland for toxin analysis.

The following tests were performed in the first toxin study:

A – 2 plates MEA untreated control (spores rubbed and extracted)
B – 2 plates MEA 100% SNL treated (spores rubbed and extracted)
C – 2 plates MEA untreated control (entire agar blended and extracted)
D – 2 plates MEA 100% SNL treated (entire agar blended and extracted)
E – 2 drywall circles untreated control
F – 2 drywall circles 100% SNL treated

Dr. Jarvis recommended that the study be repeated using Uncle Ben's Rice to promote superior toxin production. The rice was prepared according to the manufacturer's directions. A 100-gram sample of rice was placed into 3 separate sterile beakers. A 10 mL inoculum of the stock *Stachybotrys* spore suspension was stirred into each beaker of rice. The rice was incubated at 25°C for 10 days. The rice was then treated with 100 mL of the SNL for 10 minutes and drained. The rice was then washed twice with 100 mL of sterile water and drained. An aliquot of 100 mL of 95% ethanol was added to the rice and stirred for 10 minutes. The ethanol was poured into centrifuge tubes and spun at 15,000 rpm for 15 minutes. The supernate was transferred to a clean tube and sealed. The tubes were again sent to Dr. Jarvis at U of M.

The following tests were performed in the second toxin study.

G – untreated rice control, flooded with 100 mL sterile water
H – rice treated with 100 mL of 100% SNL
I – rice treated with 100 mL of 10% SNL

Results

Table #1: SNL Disinfection Efficacy against *Stachybotrys chartarum*

Concentration	Rep.	Count/ mL	Average $\log_{10}$ count	Log reduction	% Reduction
Stock	A	7,500,000			
	B	7,300,000	6.869		
50% MDF	A	<1			
	B	<1	0	>6.869	99.999987
10% MDF	A	<1			
	B	<1	0	>6.869	99.999987
1% MDF	A	540,000			
	B	500,000	5.716	1.153	92.97
0.1% MDF	A	6,500,000			
	B	5,900,000	6.792	0.077	16.22
0.01% MDF	A	7,800,000			
	B	6,900,000	6.866	0.003	0.68

Table #2: *Stachybotrys* Toxin Neutralization by MDF

Test	% SNL	Result
A	0	No toxin detected
B	100	No toxin detected
C	0	No toxin detected
D	100	No toxin detected
E	0	Stachybotrys toxins detected
F	100	No toxin detected
G	0	Stachybotrys toxins detected
H	100	No toxin detected
I	10	No toxin detected

Discussion

Under laboratory conditions the MDF kills 99.99999% (>6.89 $\log_{10}$) of *Stachybotrys chartarum* spores at dilutions down to 10%. This exceeds the standard EPA requirement of a 6-log reduction to demonstrate high-level disinfection. The killing efficacy drops dramatically below the 10% concentration with essentially no effect below 1%.

The preliminary toxin neutralization studies demonstrated promise but will require further studies to prove efficacy. According to Dr. Jarvis, MEA does not provide the adequate precursors for Stachybotrys to produce toxins. The toxins were, however, produced in the drywall samples. Treatment of the drywall samples and the rice by the MDF formula eliminated the toxins from the material. This particular strain of *Stachybotrys chartarum* did not demonstrate the genetic ability to produce the entire range of thichothecene and sterigmatocystine toxins. More work may be necessary to demonstrate efficacy on these compounds.

Conclusions

The MDF will kill 99.99999% (>6.89 $\log_{10}$) of *Stachybotrys chartarum* spores under laboratory conditions. The formula was also found to neutralize *Stachybotrys* toxins when the organism was grown on rice and wet drywall.

Future studies should include efficacy tests in naturally flooded environments. Toxin analysis should include the mycotoxins produced by other genera of molds.

Douglas A. Rice
Laboratory Director
Environmental Health Services – Colorado State University