Effectiveness of Vital Oxide for Controlling Fungal Contamination on Building Materials

Estelle Levetin, PhD

Faculty of Biological Science, The University of Tulsa

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Abstract

Rationale: Mold growth on building materials can be an exposure risk for atopic individuals. The gas chlorine dioxide is an effective fungicide but is unstable. Vital oxide (VO) is an aqueous solution containing either 0.2% or 0.5% chlorine dioxide in a stabilized form. The goal of this investigation was to determine the effects of VO on fungi commonly found growing on building materials.

Methods: Ceiling tile and sheetrock squares (36 cm^2) were sterilized and then saturated with either sterile distilled water or various concentrations of VO. Saturated squares were inoculated with a spore suspension of one of the following fungi: *Alternaria alternata, Aspergillus fumigatus, Aspergillus versicolor, Chaetomium globosum, Penicillium* sp., and *Stachybotrys chartarum.* Cultures were incubated at room temperature for up to eight weeks. Spray applications of VO were also tested on ceiling tile squares inoculated with *Asp. fumigatus* or *S. chartarum*. Spore germination of all six species was evaluated in Sabouraud's broth with and without VO for 96 hrs.

Results:VO solutions containing either 0.1% or 0.2% chlorine dioxide inhibited growth of all six fungi on both ceiling tile and sheetrock squares. Spray applications were also effective for the species tested. In the germination experiments, some spore germination occurred in the medium with 0.1% chlorine dioxide ranging from <1% germination for *Alternaria* spores to 18% for *Chaetomium* spores; however, media with higher chlorine dioxide levels prevented spore germination for all species.

Conclusions:VO inhibited growth of fungi on sheetrock and ceiling tiles and shows possible applications for control of indoor fungal contamination.

Introduction

- Water-damaged building materials, such as ceiling tiles and sheetrock, are prone to fungal contamination due to their high cellulose content. Fungal growth on these materials can be an exposure risk for mold sensitive individuals.
- The gas chlorine dioxide is an antimicrobial pesticide known for its disinfectant properties for the past century. This gas is an effective fungicide but requires special handling. It has been unstable in liquid and is often prepared on-site when liquid applications are needed.
- Vital oxide (VO) is an aqueous solution containing either 0.2% or 0.5% chlorine dioxide in a stabilized form. The current project was undertaken to determine the effectiveness of VO for controlling fungal growth on building materials.

Methods and Materials

- **Vital Oxide**: Two preparations of Vital Oxide (VO) were used in these experiments: Ready To Use (VO-RTU) with 0.2% ClO_2 and Professional Strength (VO-PS) at 0.5% ClO_2
- **Spore germination**: Spore suspensions of *Alternaria alternata, Aspergillus fumigatus, Aspergillus versicolor, Chaetomium globosum, Penicillium* sp., and *Stachybotrys chartarum* were prepared by harvesting spores from cultures grown on malt extract agar. Spore germination of all six species was evaluated in Sabouraud's broth with and without VO.
- **Building material cultures**: Ceiling tile (CTS) and sheetrock (SRS) squares (36 cm^2) were sterilized by autoclaving and then saturated with either sterile distilled water or various concentrations of VO. Saturated squares were inoculated with a spore suspension of test fungi and incubated at room temperature for up to 8 weeks.
- **Spray application of VO**: Sterile CTS and SRS were saturated with sterile distilled water and inoculated with test fungi. When growth was visible, half the materials were sprayed with 5 sprays (3.5 ml) VO. Incubation continued for up to 10 wks.
- **Viability Tests**: Sterile SRS were saturated with sterile distilled water and inoculated with *Stachybotrys chartarum* or *Aspergillus fumigatus* and incubated 2 to 4 wks. One half of the cultures were sprayed with 7 sprays (5 ml) of VO-RTU. SRS were incubated for an additional 48 hrs. Spores were harvested with a cell lifter and placed in 10 ml of sterile water. Spores were counted with a hemacytometer and percent viability was determined by dilution plating.

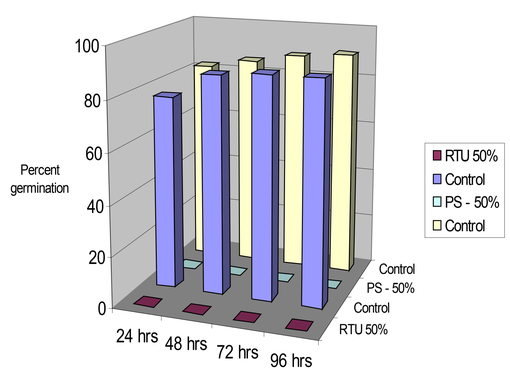
Results

- Culture medium with 50% VO-RTU was effective in inhibiting spore germination for *Alternaria*, *Stachybotrys*, and *Penicillium* spores. *Chaetomium* spores had an 18% germination rate. The medium with 50% of VO-PS was effective in inhibiting germination for all spores.
- Pretreatment of CTS and SRS with VO-RTU was effective in preventing fungal growth. In fact, CTS were still able to inhibit fungal growth 7 months after saturation.
- Spray applications using VO-RTU on CTS and SRS with active fungal growth resulted in a resumption of fungal growth within approximately 2 wks when CTS or SRS were water saturated and not allowed to dry.
- Spray applications using VO-PS were more effective inhibiting fungal growth up to 2 months for some fungi even in water saturated conditions.
- Spray applications of VO-RTU were effective in reducing viability of *Aspergillus fumigatus* spores.

Fungus	Mean percent spore germination after 96 hours	
	Control	50% RTU
Alternaria alternata	>90	0
Chaetomium globosum	>90	18.3
Stachybotrys chartarum	88.3	0
Penicillium sp.	>90	0

Fungus	Mean percent spore germination after 96 hours	
	Control	50% PS
Alternaria alternata	88.3	0
Aspergillus versicolor	76.7	0
Aspergillus fumigatus	60.0	0
Chaetomium globosum	86.7	0
Penicillium sp.	>90	0
Stachybotrys chartarum	>90	0

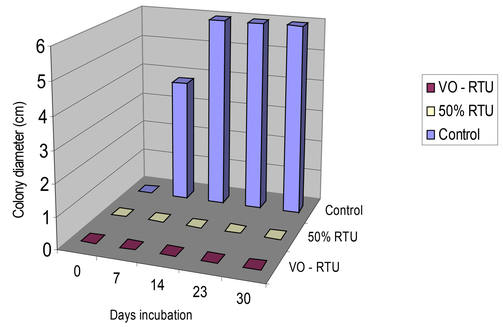
Percent germination of *Stachybotrys* spores



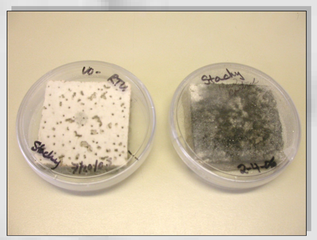
Fungi	Mean colony diameter (cm) of fungi after 30 days incubation on CTS saturated with VO or water		
	Control	VO - RTU	50% RTU
Alternaria alternata	>6.0	0	0
Aspergillus fumigatus	>6.0	0	0
Aspergillus versicolor	4.0	0	0
Chaetomium globosum	0.23*	0	0
Penicillum sp	5.6	0	0
Stachybotrys chartarum	>6.0	0	0

*Growth of *Chaetomium* on ceiling tiles could only be detected after perithecia formed. After 8 weeks perithecia were visible over an area approximately 4 cm in diameter.

Average colony diameter of *Aspergillus fumigatus* growing on ceiling tiles saturated with VO or sterile water



Fungi	Mean colony diameter (cm) of fungi after 30 days incubation on SRS saturated with VO or sterile water		
	Control	VO - RTU	50% RTU
Alternaria alternata	>6.0	0	0
Aspergillus fumigatus	>6.0	0	0
Aspergillus versicolor	3.6	0	0
Chaetomium globosum	>6.0	0	0
Penicillum sp.	>6.0	0	0
Stachybotrys chartarum	>6.0	0	0



Pretreatment of ceiling tiles with VO-RTU inhibited growth of *Stachybotrys* after 7 months following treatment. Ceiling tile saturated with VO-RTU in July 2005 was inoculated with *Stachybotrys* spore suspension on 4 Feb 2006. Control tile was inoculated at the same time.

Colony diameter of various fungi growing on saturated SRS. Treated squares were sprayed with VO-PS 2 to 3 weeks after inoculation. Final measurements and observations were made 5 to 7 weeks after spraying

	Control SRS	SRS sprayed with VO-PS
Alternaria alternata	6.0 cm	6.0 cm – Actively growing but less dense than controls
Aspergillus fumigatus	4.6 cm	No measurable colonies but one culture had small areas of growth
Aspergillus versicolor	5.32 cm	4.50 cm – Sprayed areas appear dead.
Chaetomium globosum	6.0 cm	1.85 cm – Sprayed areas appear dead.
Cladosporium cladosporoides	4.5 cm	3.67 cm – Sprayed areas appear dead.
Penicillium sp.	4.7 cm	No visible growth

Viability of *Aspergillus fumigatus* following spray application of VO-RTU

	Harvested conidia x 10^6	CFU x 10^6	Viability
Control 1	28.0	14.4	51.4%
Control 2	14.0	11.8	84.3%
Control 3	12.5	11.4	91.2%
VO-RTU Spray 1	27.5	0.34	1.24%
VO-RTU Spray 2	24.8	0.05	0.20%
VO-RTU Spray 3	36.3	0.21	0.58%

Conclusions

- VO inhibited spore germination of test fungi
- Pretreatment with VO inhibited the growth of fungi on sheetrock and ceiling tiles
- Spray applications of VO-PS were effective in stopping fungal growth for several weeks even in saturated conditions
- More work needs to be done to determine the most effective spray application methods for VO-RTU

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