Natural products as antifungal agents in carpets

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ABSTRACT
The purpose of this research was to identify non-toxic, natural products that could act as substitutes for hazardous antifungal agents currently applied to carpets, and to investigate the efficacy of ethylcellulose as an extender of these products in carpets. Ten natural products were tested against *Aspergillus versicolor* or *Penicillium chrysogenum*-inoculated agar plates, the zones of inhibition observed, and thymol, benzoate and sorbate (TBS) chosen as most effective. Different combinations of these three agents were applied to fungal-inoculated carpet squares, and spore number data indicated an optimum mixture of 0.6% thymol, and 3% each of sorbate and benzoate. Ethylcellulose was then added to the TBS mixture as a coating/binding polymer (TBSC), to improve stability. TBSC- and TBS-treated carpet squares were compared and results indicated that the addition of the cellulose maintained the antifungal properties of the TBS even in carpet samples stored for up to 9 months.

INDEX TERMS
Carpet; Fungi; Source control; Preventive

INTRODUCTION
Fungal growth in building materials such as ceiling tile, drywall and carpet has become a major concern in indoor problem environments (Godish, 2001). Carpets in particular are a problem because they are in such widespread use and because foot traffic and spills provide nutrient sources that, along with moisture, provide an excellent medium for mould growth. The application of antimicrobial agents to carpets began in the 1990s (Broughton *et al.*, 1999), but such agents are considered relatively toxic. Although a relatively large body of literature exists describing the antifungal properties of a number of low toxicity, natural products (Hunter and Sege, 1973; Davidson *et al.*, 1983; Hitokoto *et al.*, 1998), these types of food or plant extracts have never been applied to carpets.

The purpose of this study is to investigate the efficacy of 10 natural products as antifungal agents for commonly occurring indoor fungal contaminants such as *Aspergillus* sp. and *Penicillium* sp. Ten natural products were first tested in a Petri dish environment. Then, the top three agents were tested separately and in combination, at different concentrations, on fungal-inoculated carpet squares. After an optimum mix and concentrations were determined, a cellulosic binder was added to determine its affect on the longevity of the antifungal properties.

METHODS
Aspergillus versicolor and *Penicillium chrysogenum* were isolated from an indoor environment. Spore suspensions of each fungus, containing 2.0×10^5 spores/ml, were prepared and a 0.5 ml aliquot was spread onto Potato Dextrose Agar (PDA) plates. Six millimetre filter disks were saturated with appropriate concentrations of each antifungal agent solution and placed onto the centre of fungal-inoculated PDA plates. After incubation at 25°C for 4 weeks, zones of inhibition around the disks were observed and measured.

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Carpet (tufted synthetic carpet with synthetic backing) was cut into 3 cm × 3 cm squares and were treated with different combinations and concentrations of thymol, benzoate and sorbate. Spore suspensions of each fungus, containing 2.0×10^3 spores/ml in a 0.1% Potato Dextrose Broth solution, were prepared and a 0.5 ml aliquot was inoculated onto both treated and untreated carpet squares. Samples were placed in static test chambers to maintain a stable relative humidity (RH) level of 97% at 25°C. After 7 and 14 days of incubation, spores were extracted from the carpet samples, and spore numbers were determined with a hemocytometer.

Carpet squares treated with and without the addition of a cellulose binder mixed with the antifungal agents were stored up to 9 months to examine the effect of the binder on longevity of antifungal activity.

RESULTS

Of the 10 natural products tested, cinnamaldehyde, eugenol, trans-anethole, thymol, benzoate and sorbate inhibited the growth of both fungi (Table 1). Of these, thymol, benzoate and sorbate were selected for further study. Table 2 shows the effect of various concentrations of thymol, benzoate and sorbate only and in combination. The Minimum Inhibitory Concentration (MIC) from the Petri dish assay was 0.5% thymol, 2.5% benzoate and 5% sorbate.

Table 1 Zone of inhibition of antifungal agents against *A. versicolor* and *P. chrysogenum*

Natural agents	Fungi	
	A. versicolor	*P. chrysogenum*
Tea-tree oil (20%, v/v)[a]	Not inhibited	Not inhibited
Green tea extract (20%, w/v)	Not inhibited	Not inhibited
Garlic oil (2%, v/v)	Not inhibited	Not inhibited
Benzoate (15%, w/v)	27.7 ± 0.67 mm[b]	29.7 ± 0.33 mm
Sorbate (15%, w/v)	30.7 ± 0.67 mm	30.3 ± 0.88 mm
Propionate (15%, w/v)	Not inhibited	Not inhibited
Thymol (5%, w/v)	Completely inhibited	Completely inhibited
Trans-anethole (1%, v/v)	Mostly inhibited[c]	23.3 ± 1.67 mm
Cinnamaldehyde (5%, v/v)	Mostly inhibited	Completely inhibited
Eugenol (5%, v/v)	Completely inhibited	Completely inhibited
Ethyl alcohol (5%, v/v)[d]	Not inhibited	Not inhibited
Distilled water[d]	Not inhibited	Not inhibited
Triclosan (0.15%, w/v)[e]	Mostly inhibited	24.7 ± 2.67 mm

[a]Concentration of agents (vol/vol % or wt/vol %).

[b]Zone of inhibition: the diameter of a circle of growth inhibition around the filter paper disk (mean ± SE, in mm).

[c]Zone of inhibition was not circlular, thus not measurable; however, fungal coverage was less than 10% of the area of the Petri dish.

[d]Tested as a control.

[e]Tested as a commercially available toxic antifungal agent, for comparison.

Table 2 Zone of inhibition and optimum concentration of serial concentrations of selected antifungal agents against *A. versicolor* and *P. chrysogenum*

Agents	Concentration[a] (%)	Fungi	
		A. versicolor	*P. chrysogenum*
Benzoate	1	Not inhibited	Not inhibited
	2.5[b]	6 ± 6.33[c]	17 ± 0.58
	5	13 ± 6.67	19 ± 0.88
	7.5	22 ± 1.53	18 ± 1.00
	10	24 ± 0.33	23 ± 1.45
	12.5	22 ± 0.00	27 ± 0.67
	15	28 ± 2.73	26 ± 2.33
Sorbate	1	Not inhibited	Not inhibited
	2.5	Not inhibited	22 ± 0.00
	5[b]	17 ± 0.33	22 ± 0.88
	7.5	23 ± 2.60	24 ± 1.00
	10	20 ± 3.06	24 ± 0.67
	12.5	30 ± 0.58	24 ± 0.33
	15	29 ± 0.67	34 ± 0.33
Thymol	0.1	Not inhibited	Not inhibited
	0.25	Not inhibited	15 ± 0.58
	0.5[b]	17 ± 0.00	21 ± 0.88
	0.75	17 ± 0.33	27 ± 0.58
	1	Completely inhibited	23 ± 1.20
	3	Completely inhibited	Completely inhibited
	5	Completely inhibited	Completely inhibited

[a]Concentration is expressed as %, wt/vol; 0.5 ml of each solution added to Petri dish.
[b]Minimum inhibitory concentrations selected: benzoate—2.5%, sorbate—5%, thymol—0.5%.
[c]Zone of inhibition: the diameter of a circle of growth inhibition around the filter paper disk (mean + SE, in mm).

Test results confirmed that untreated carpet squares inoculated with 1000 spores increased to 6×10^7 spores within 1–2 weeks of incubation. As with the Petri dish assay results, thymol, benzoate and sorbate individually controlled the growth of *A. versicolor* and *P. chrysogenum* on treated carpet squares (Figure 1). Thymol was extremely effective, with an inhibition percent, compared to untreated controls, of 90% after 30 days of incubation, while sorbate and benzoate were less effective, at 65 and 58%, respectively.

In combination, a mixture of 0.6% thymol, 3% benzoate and 3% sorbate was effective at controlling the growth of *A. versicolor* (Figure 2), and *P. chrysogenum* (data not shown due to similar results), with an inhibition percent, compared to untreated controls, of 99%.

Finally, carpets treated with ethylcellulose in combination with the TBS were compared to carpets treated with the TBS only. When treated carpet samples were stored for up to 6 months prior to inoculation and incubation for 7 and 14 days, both the TBS and the TBSC carpet samples had inhibition percents of 99% for both fungi. However, after 9 months of storage, the TBSC inhibition percent remained at 99%, while the TBS treatment inhibition percent dropped slightly to 98% for *A. versicolor* and 96% for *P. chrysogenum*.

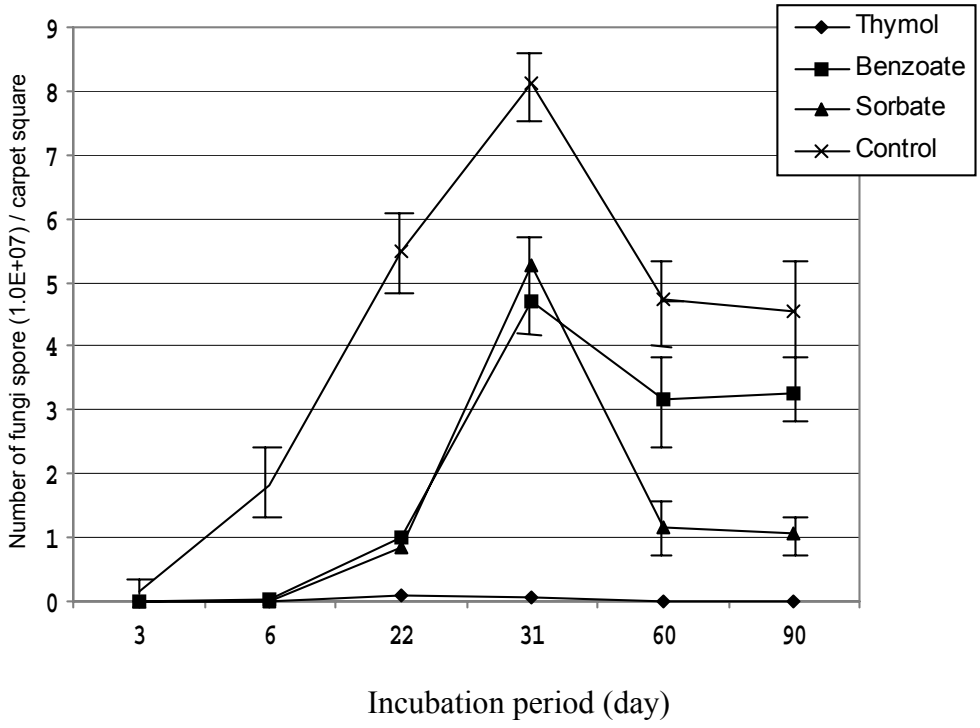

Figure 1 Inhibitory effects of 1% thymol (5.5 µg/mm^2), 5% benzoate (27.8 µg/mm^2) and 5% sorbate (27.8 µg/mm^2) on carpet squares (900 mm^2) against *A. versicolor*.

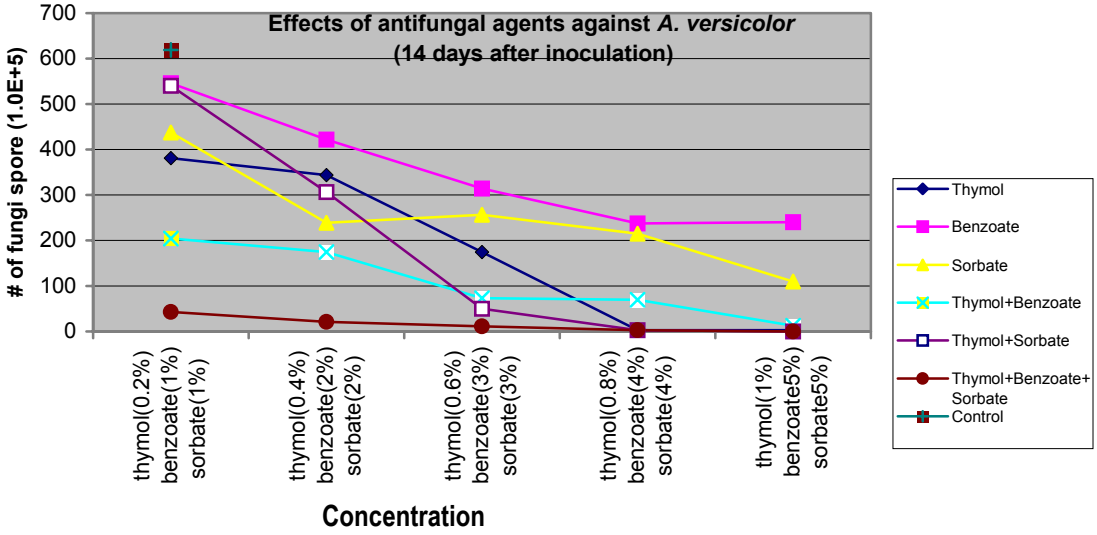

Figure 2 Effects of antifungal agents against *A. versicolor* at various concentrations and combinations on carpet squares.

DISCUSSION

In the Petri dish assay, eugenol, cinnamaldehyde trans-anethole, thymol, benzoate and sorbate all showed excellent antifungal activity. However, eugenol, cinnamaldehyde and trans-anethole are sensitive to prolonged exposure to air and light. Thus, thymol, benzoate and sorbate were the three chemicals taken from the Petri dish stage to the carpet sample phase of the study. They performed well individually, with thymol the most effective, at an inhibition percent of 90% for a 1% solution. Even though benzoate and sorbate did not perform as well individually, when all three were combined, the inhibition percent rose to 99%, even though

the concentration of each of the three agents in the combined solution was reduced, from 1, 5 and 5% to 0.6, 3 and 3%, for thymol, benzoate and sorbate, respectively.

Thus, while thymol was the most effective agent individually, the synergistic combination of the three agents allowed for the use of reduced concentrations while still achieving optimum fungal control. This can have an important impact on the economic feasibility of adding these compounds to building materials. The other important factor that will add to this fungal control approach is the effectiveness of the materials over extended time periods, especially when the ethylcellulose extender is added, as it maintained the performance of the three agents to at least 9 months.

CONCLUSIONS AND IMPLICATIONS

- Of the 10 agents tested, thymol, benzoate and sorbate were overall the most effective antifungal agents.
- The Minimum Inhibitory Concentrations of the above three agents were found to be 0.5, 2.5 and 5%, respectively.
- Thymol, benzoate and sorbate (TBS) combined in a 0.6, 3, 3% mixture had the optimum synergistic effectiveness in inhibiting the growth of *A. versicolor* and *P. chrysogenum* on carpet squares.
- The addition of ethylcellulose to the TBS mixture extended the antifungal effectiveness for at least 9 months.
- Implications for application to carpet to significantly reduce potential fungal growth in indoor environments are promising.
- Implications for application to other building materials are likewise as promising.

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