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Copper oxide-impregnated fabrics for the control of house dust mites

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Copper oxide (CuO) has broad-spectrum anti-microbial and anti-fungal properties. The aim of this study was to test the acaricidal efficacy of CuO-impregnated fabrics on the common house dust mite, *Dermatophagoides farinae*. The overall vitality/mobility of the mites was reduced when they were exposed to the CuO-impregnated fabrics and, when possible, the dust mites migrated to fabrics where no CuO was present. The mortality of mites exposed for 10 days to fabrics containing 0.2% (w/w) CuO was significantly higher than the mortality of mites on control fabrics (72 $\pm$ 4 and 18.9 $\pm$ 0.3%, respectively). The mortality reached 95.4 and 100% with fabrics containing 0.4 and 2% CuO after 47 and 5 days, respectively. The acaricidal effect of copper oxide seems to be due to direct toxicity. The use of fabrics containing copper oxide may thus be an important avenue for reducing house dust mite populations, and for reducing the load of dust mite allergens.

Keywords: acaricide; house dust mites; allergy; copper oxide; fabrics; *Dermatophagoides farinae*; control

1. Introduction

It is estimated that 15% of the Western general population suffer from one or more allergic disorders of which allergic rhinitis is the most common (Skoner 2001). The latter condition affects an estimated 20–40 million people in the USA alone. Similarly, nearly 15 million Americans, including almost 5 million children, have asthma. Approximately 5500 persons die each year from asthma (Redd 2002).

House dust mites (HDM) are considered to be an important source of allergen implicated in allergic asthma, rhinitis, conjunctivitis and dermatitis (Brunton and Saphir 1999). HDM are microscopic arthropods belonging to the family Pyroglyphidae of the order Acarina. *Dermatophagoides pteronyssinus* Trouessart 1897 and *Dermatophagoides farinae* Hughes 1961 are the most common HDM species worldwide. Bedding, carpets and sofas are the main biotopes for HDM within the human habitations. HDM feed mainly on human skin scales and microorganisms found on the scales. The skin scales have to be pre-digested by fungi such as *Aspergillus* (Van Bronswijk et al. 1987). Mite allergens are excreted together with the faeces, which are further decomposed by microorganisms and become airborne. The allergens excreted from dust mites are responsible for allergic reactivity in many people (Milian and Diaz 2004; Fernandez-Caldas and Iraola 2005). The higher the allergen levels, the greater are the clinical symptoms (Custovic et al. 1996; Taggart et al. 1996). Reduction of HDM populations and allergens would thus significantly reduce the allergenic load and consequently reduce the severity of symptoms. As a result, there will be an improvement in the quality of life for those suffering from dust mite-related allergies.

High concentrations of copper oxide (CuO) are toxic to micro-organisms (Borkow and Gabbay 2005). Copper ions, either alone or in copper complexes, have been used for centuries to disinfect liquids, solids and human tissue. Today, soluble copper is used as a water purifier, algaecide, fungicide, nematocide, molluscicide, bactericide and anti-fouling agent (Borkow and Gabbay 2005). Studies have also shown that copper has insecticidal (Servia et al. 2006) and acaricidal properties (Filser et al. 2000).

Based on copper's acaricidal and antifungal properties, we hypothesized that CuO-impregnated fabrics could kill HDM directly and/or indirectly by destroying fungi such as *Aspergillus*. The aim of this study was to test the efficacy of CuO-containing fabrics on the vitality and viability of HDM *in vitro*.

2. Materials and methods

2.1. Test fabrics

Cellulosic fibres were permanently plated with CuO as described by Borkow and Gabbay (2004) and Gabbay et al. (2006). Fabrics were made with

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cellulosic fibres of which 10, 20 or 100% of the fibres were plated with CuO (Figure 1). The fabrics were examined by scanning electron microscopy (SEM; Jeol JMS 5410 LV scanning electron microscope, Japan) and the presence of copper content was determined by X-ray photoelectron spectrum analysis (Link IV, ISIS, Oxford Instruments, England). The copper content of the fabrics was determined by using inductively coupled plasma atomic emission spectrometry (Spectroflame modula E from Spectro GMBH, Kleve, Germany). The fabrics contained 0.2, 0.4 or 2% (w/w) CuO, respectively. Similar fabrics but without CuO were used as controls.

2.2. *Antifungal efficacy of the test items*

Swatches of CuO-containing fabrics and control fabrics were tested for antifungal efficacy against *Aspergillus niger* van Tieghem 1867*, Trichophyton mentagrophytes* Blanchard 1896 and *Candida albicans* [Berkhout 1923] using the American Association of Textile Chemists and Colorists (AATCC) Test Method 100-1993. Briefly, sterile swatches weighting 0.50 ± 0.01 g of both CuO-containing fabrics and control fabrics were placed in sterile vials and exposed to 0.5 mL solutions containing 1×10^5 to 4×10^6 colony forming units (CFU). The samples were then incubated at room temperature for 24 h. After the incubation periods, the test samples were transferred aseptically to 250-mL jars, and 100-mL Letheen Broth were then immediately added. The jars were sealed tightly and shaken vigorously for 1 min. Serial 10-fold dilutions with water were made and 1-mL aliquots were plated by standard bacteriological procedures, in duplicate, on Tryptic Soy Agar plates. The plates were incubated at 35 ± 1°C for 24 h and the numbers of fungi colonies were determined by the standard Pour Plate Count. These tests were carried out by FDA-approved laboratories: Amino-Lab Laboratory Services, Weizmann Industrial Park, Nes Ziona, Israel, and NAMSA, Irvine, CA, USA.

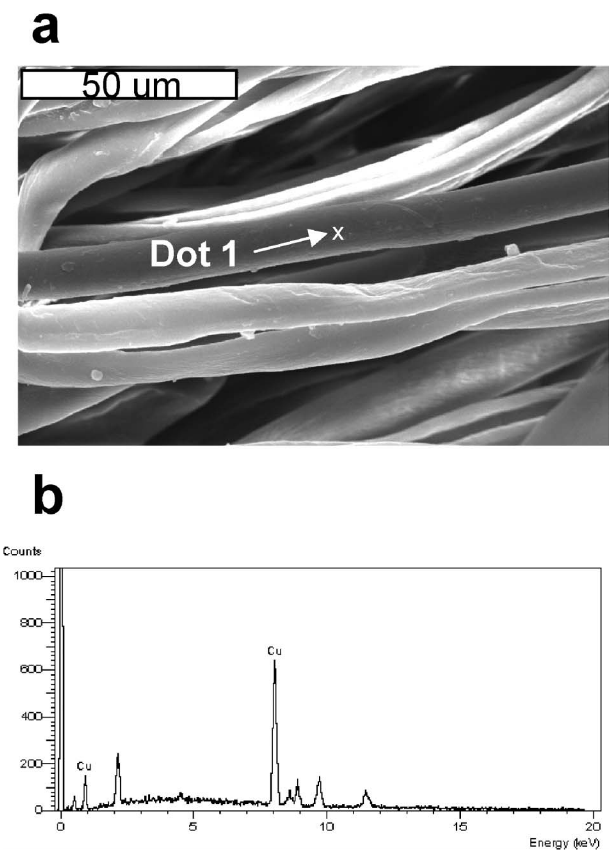

Figure 1. Scanning electronic microscope pictures of CuO-plated cellulosic fibres. (a) Mixture of CuO-plated fibres and untreated fibres. The X-ray photoelectron spectrum analysis of dot 1 on a plated fibre in (a) is shown in (b).

2.3. *Mites*

Dermatophagoides farinae was cultured in the laboratory using a mixture of horse dander/medical yeast (2:1) at a temperature of 25 ± 1°C and 75 ± 5% RH.

2.4. *Acaricidal efficacy*

CuO-containing fabrics and control fabrics (3.5 cm in diameter) were introduced into a micro-titre plate with the same diameter and glued to the bottom of the vial with regular plastic glue (White Glue, Blanbang, China). After 24 h, 100–200 mites of all developmental stages and 10 mg of culture medium were placed on the fabrics. The opening of each vial was sealed with non-hardening glue, which prevented the mites from escaping. During the entire experiment, the mites were incubated under the above-described conditions. The mobility of mites; their egg-laying activity; appearance of a new generation of HDM; and the mortality of the HDM was monitored and rated as 'almost all alive'; 'some dead'; 'most dead' and 'all dead' every 2–4 days under a stereo-microscope. When necessary, additional food was added for the surviving mites. At the end of each experiment, each vial and the fabric within was rinsed thoroughly with 40% alcohol. The liquid was then filtered through a filter paper (7 cm diameter) and the mites (dead and alive) were counted under the microscope. The rinsed vials and fabrics were re-examined under a stereo-microscope for any remaining mites. Each experiment was conducted in triplicate.

2.5. *Statistical analysis*

The percent of fungal reduction was determined according to the following formula: $100(A–B)/A = \%R$ and the log reduction was calculated by the following formula: Log *A*–Log *B* = Log *R* to the nearest hundred, where *R* is the reduction; *A* is the CFU of the challenge organism; and *B* is the CFU recovered from the inoculated test sample. Student's *t*-test was performed using SigmaPlot 9.0 (Jandel Corporation, USA).

3. Results

3.1. *Biocidal properties of the CuO-containing fabrics*

Over 99% of *C. albicans*, *T. mentagrophytes* and *A. niger* fungi were killed by fabrics containing 0.2% CuO. No reduction in antifungal efficacy occurred even after 50 home washes of the CuO-containing fabrics. In contrast, significantly lower reduction of fungal titres occurred in fabrics that did not contain CuO (11 $\pm$ 5.5, 33 $\pm$ 10 and 9.2 $\pm$ 16% for *C. albicans*, *A. niger* and *T. mentagrophytes*, respectively; $P < 0.001$).

3.2. *Acaricidal properties of the CuO-containing fabrics*

Four kinds of experiments were conducted in order to test the acaricidal efficacy of CuO-containing fabrics.

3.2.1. *Experiment I*

Mites were exposed to swatches of fabrics containing 0.2% CuO and compared to control fabrics. After 4 days of culture, the mites exposed to the copper-containing fabrics showed a very low activity while those on the control fabrics were very active. After 10 days, the mean percent mortality of mites exposed to 0.2% CuO-containing fabrics was 72 $\pm$ 4%, while the mortality on the control swatches was 18.9 $\pm$ 0.3% ($P < 0.001$).

3.2.2. *Experiment II*

Mites were exposed to fabrics containing 0, 0.4 and 2% CuO. As depicted in Figure 2, most mites were dead after 1 day of exposure to 2% CuO and all were dead after 5 days. Most mites exposed to 0.4% CuO-containing fabrics died after 12 days and the mortality was 95.4% at day 47. HDM on control patches showed a significantly lower mortality of 32.5% at day 47 ($P < 0.01$). In the control fabrics the mites were very active, and mating and egg-laying mites could be observed everywhere, while those exposed to CuO-impregnated fabrics showed a low activity and few eggs were laid during the period of the experiment.

3.2.3. *Experiment III*

Mites were exposed to two round fabric swatches glued to each other in which the lower swatch was a 35-mm (diameter) untreated fabric and the upper swatch was either a 20- or 35-mm; 0.4 or 2% CuO-containing fabric; a 35-mm silver nitrate (SN)-containing fabric or 35-mm untreated control fabric (Figure 3). All mites exposed to the test items containing 2% CuO, or to the 35-mm 0.4% CuO, were dead after 5 days of exposure. Almost all mites exposed to the 20-mm 0.4% CuO fabric were dead at day 12 and most of those which survived were concentrated in the untreated area. In contrast, the mites exposed to 2% SN or control fabrics were very active and $<5\%$ of the mites were dead at 12 days of exposure (Figure 3). At day 47, a total of 100, 37 and $<5\%$ of the mites exposed to the 20-mm 0.4% CuO, 2% SN and control fabrics were dead, respectively.

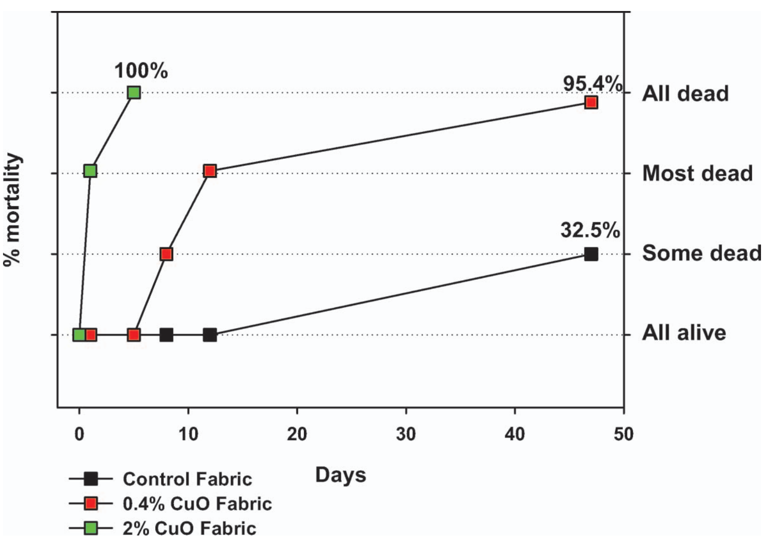

Figure 2. Dose-dependent acaricidal activity of 0.4 and 2% CuO-containing fabrics.

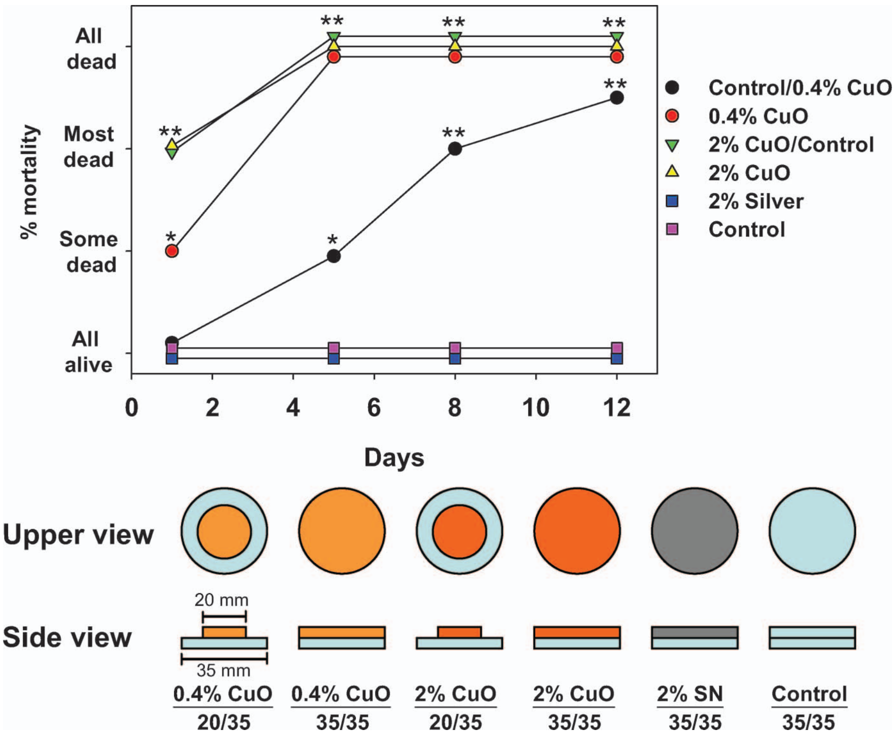

Figure 3. Survival of *D. farinae* exposed to fabrics containing CuO or SN. The statistical difference between each group and the control fabric was determined using Student's *t*-test. $*P < 0.05$; $**P < 0.01$.

3.2.4. Experiment IV

The efficacy of a tri-laminated fabric (composed of an external layer of 0.4% CuO, an internal layer of a breathable barrier membrane, made from a polyether-based polyurethane thermoplastic material and a third layer made of cotton sheeting) was compared to 0.2% CuO-containing and control fabrics (Figure 4). A plastic eraser was placed on top of each two fabrics to allow a better contact between the mites and the fabrics. After 10 days, 100 and 99% of mites placed between the impregnated sides of two tri-laminated fabrics or between two 0.2% impregnated fabrics died, respectively. Mites exposed between the impregnated and non-impregnated sides of two tri-laminated fabrics showed a mortality of 87.2%, while those exposed to the control fabrics had a mortality of only 42.5% after 26 days of exposure ($P < 0.01$; Table 1).

4. Discussion

Our study shows that CuO-containing fabrics significantly control or eliminate the population of HDM. When the mites are in direct contact with a high enough concentration of copper-containing fabrics and without access to untreated areas, they died within a few days.

The negative effect of high copper concentrations on insects has been demonstrated by an increasing delay of larval growth of the midge *Chironomus riparius* Meigen 1804 (Servia et al. 2006), of the coccinellid beetle *Olla v-nigrum* [Mulsant 1866] (Michaud and Angela 2003) and of the mosquito *Aedes albopictus* [Skuse 1894] (Bellini et al. 1998). The acaricidal effect of copper has also been described in a re-colonization experiment in which the abundances of gamasid mites were significantly higher in copper-uncontaminated soil (Filser et al. 2000). Similarly, the densities of gall mites in two birch species (*Betula pubescens* Ehrh. and *B. pendula* Roth) were found to be negatively correlated with the levels of copper in the air in the area near to a copper–nickel smelter (Koricheva et al. 1996). Interestingly, copper ions inhibit an elastase-like enzyme present in *D. pteronyssinus* and *D. farinae* (Stewart et al. 1994).

In contrast to the high susceptibility of microorganisms to copper, human skin is not sensitive to copper and the risk of adverse reactions due to dermal exposure to this metal is extremely low (Hostynek and Maibach 2003; Gorter et al. 2004). Copper is considered safe to humans, as demonstrated by the widespread and prolonged use by women of copper intrauterine devices (Hubacher et al. 2001; Anon. 2002; Bilian 2002). Furthermore, copper is an essential metal needed for normal metabolic processes. The National Academy of Sciences Committee established the US recommended Daily Allowance of 0.9 mg of copper for normal adults. This committee also noted that daily intakes

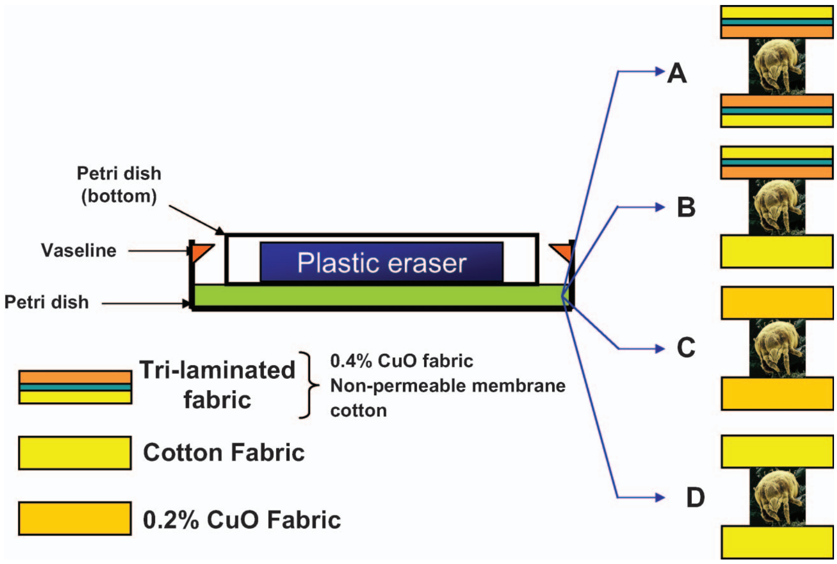

Figure 4. Survival of *D. farinae* exposed to tri-laminated fabrics.

Table 1. Percentage mean mortality of mites placed between impregnated and non-impregnated CuO fabrics.

Test group	Day					
	6	10	13	17	23	26
A	75[a]	100[b]				
B	0[a]	25[a]	25[a]	50[a]	75[a]	87.2[b]
C	25[a]	99.1[b]				
D	0[a]	0[a]	0[a]	25[a]	25[a]	42.5[b]

[a]Estimated. [b]Exact count.

of up to 3 mg/day in children and 8–10 mg/day for adults are considered tolerable and non-toxic (Trumbo et al. 2001).

Recently, a platform technology has been developed that incorporates CuO into textile fibres from which woven and non-woven fabrics can be produced (Borkow and Gabbay 2004; Gabbay et al. 2006). These copper-impregnated products possess broad-spectrum antimicrobial properties (Borkow and Gabbay 2004; Gabbay et al. 2006) and have been shown to possess no skin sensitization or irritation properties in animals and in humans (Borkow and Gabbay 2004; Gabbay et al. 2006). Copper, including CuO, is permitted for use in fabrics by the US Environmental Program Agency.

Based on the above, and as fungi are a key factor in the HDM food chain (Van Bronswijk et al. 1987), together with the antifungal properties of copper (Borkow and Gabbay 2005), we hypothesized that introducing CuO, a non-soluble form of copper, into fabrics, will endow them with acaricidal properties.

HDM were not severely affected by SN-impregnated fabrics. Since silver has also fungicidal properties, this suggested that the mechanism of CuO killing the mites is not necessarily via the destruction of the fungus needed to predigest the human scales, but that the CuO may have a direct toxic effect on the mites themselves. The fact that, at 2% CuO concentrations, most of the HDM died within a few days further shows that mites died as a result of the direct toxicity of CuO rather than starvation. In addition, it was observed that the overall vitality/mobility of the mites exposed to CuO-containing fabrics was much lower than in control or SN-treated fabrics and that, when possible, mites migrated to untreated parts of the test items.

The antifungal activity of copper-containing fabrics further enhances the efficacy of these fabrics to control fungi, such as *Aspergillus*, necessary for the pre-digestion of skin scales before they can be consumed by the mites (Van Bronswijk et al. 1987). In addition, the presence of fungi has been associated with asthma, cough and wheeze (Belanger et al. 2003; Su et al. 2005; Kercsmar et al. 2006). Several mechanisms for the biocidal activity of copper have been proposed. These include: denaturation of nucleic acids by binding to and/or disordering helical structures and/or by cross-linking between and within nucleic acid strands; alteration of proteins and inhibition of their biological assembly and activity; plasma membrane permeabilization; and membrane lipid peroxidation (Borkow and Gabbay 2005).

In conclusion, the results of this study clearly demonstrate that, by using CuO-containing fibres in fabrics, the HDM population can be controlled and, if enough copper is present in the fabric (0.4% and above), the mites can be eradicated. In order to assure effective killing of the HDM a CuO-containing fabric should be constructed in such a way as to prevent the mites from migrating to areas without CuO.

Obviously, in order to achieve as close as possible an allergen-free environment, all fabric material, such as those in carpets, sofas and beds, should be impregnated with CuO. By reducing the HDM population, the overall allergen levels responsible for adverse clinical reactions in allergic individuals are expected to be reduced significantly, thus improving their quality of life.

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