

The toxic effect of chitosan/metal-impregnated textile to synanthropic mites

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Abstract:

BACKGROUND: Plasma treatment enables effective binding of chitosan film to textile fibres. Heavy metal ions such as Ag⁺ adsorbed onto the chitosan coating are known to enhance toxicity to microorganisms. The acaricidal effect of chitosan and chitosan/metal adducts with Ag⁺, Zn²⁺ and Cu²⁺ was tested in laboratory experiments. Tested species *Acarus siro*, *Dermatophagoides farinae*, *D. pteronyssinus* and *Tyrophagus putrescentiae* are allergen producers and important pests in house dust, stored food and feed. The mortality was compared after 24 h of exposure of mites to plasma-treated textiles.

RESULTS: Chitosan/Ag⁺ textile caused at least 80% mortality of all species tested. Chitosan/Zn²⁺ and chitosan/Cu²⁺ textiles had a smaller effect on mite mortality than chitosan/Ag⁺. The conversion of chitosan/Ag⁺ finishing to chitosan/Ag₂O did not influence the mortality of mites in biotests, except that of *Tyrophagus putrescentiae*, where the mortality decreased from 86 to 64%.

CONCLUSION: The results support a great potential of chitosan/Ag⁺ fibres in acaricidal materials and/or mite protective food packages.

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Keywords: dust mite; allergen; control; chitosan; Ag⁺; microorganism

1 INTRODUCTION

Chitosan is a β -1,4-linked polymer of glucosamine (2-amino-2-deoxy- β -D-glucose) and lesser amounts of *N*-acetylglucosamine, formed by the deacetylation of chitin (poly-*N*-acetylglucosamine). Both chitin and chitosan are naturally occurring compounds in arthropod cuticles and the cell walls of some fungi.¹ Chitosan is a non-toxic, biodegradable polymer of high molecular weight.² It is used to prepare hydrogels, films, fibres or sponges. Most chitin-derived materials are used in the biomedical domain, for which biocompatibility is essential.¹

The beginning of the twenty-first century has seen the discovery of the antimicrobial properties of chitosan and the application of chitosan in preharvest and post-harvest protection against phytophagous fungi and bacteria.^{3,4} In addition, chitosan has shown insecticidal activities, suggesting the potential for its application against herbivorous insects.^{5–7}

Chemical modifications of chitosan have increased its toxicity to insects.⁸ Amine (–NH₂) functional groups in chitosan enhance the formation of a stable complex with metals, resulting in complexes with changed properties, including higher toxicity to pathogenic organisms.¹ Metal ions (Ag⁺, Cu²⁺, Zn²⁺, Mn²⁺ or Fe²⁺) adsorbed onto chitosan nanoparticles increase its antibacterial properties.⁹ For example Ag⁺ ions adsorbed onto chitosan nanoparticles have a bactericidal effect against *Escherichia coli*, *Salmonella choleraesuis* and *Staphylococcus aureus*. The suppressive effect of chitosan/Ag⁺ was higher than that of chlortetracycline and of chitosan itself.⁹ This indicates that the loading of metal ions into chitosan is beneficial for bacterial suppression; however, there is a lack of

information concerning the effect of chitosan/metals to arthropod pests.

Synanthropic pest mites from the group Astigmata penetrate into human habitats and persist in house dust and stored food or feed.¹⁰ Their impact as pests is via allergen production and the transport of pathogenic microorganisms.^{11,12} The control of mites is mainly based on chemical pesticides,^{13,14} and an intensive search is being carried out to find novel functional compounds.^{15–17} The next constraint on mite chemical control is legislative restriction limiting functional acaricides.¹⁸ This situation is accelerating further the research into novel compounds or protocols effectively controlling mites and their allergens.

In the present study, the acaricidal property of a chitosan complex with metal ions has been tested in laboratory experiments, with the emphasis on silver metal ions. *Acarus siro* (Linnaeus 1758), *Dermatophagoides farinae* Hughes, 1961, *D. pteronyssinus* (Trouessart, 1897) and *Tyrophagus putrescentiae* (Schränk 1781) were chosen as the model mites for experiments. The results presented confirm the great potential of chitosan/Ag⁺ textile with acaricidal properties.

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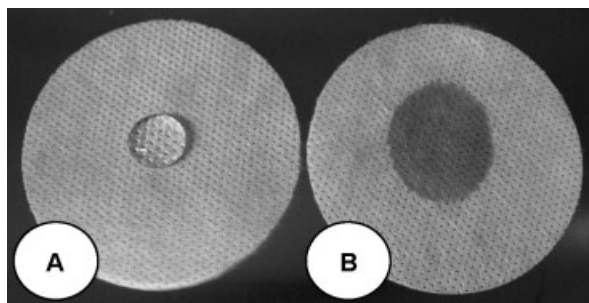


Figure 1. The improvement of chitosan gel wettability on spunbond polypropylene non-woven textile (PPNT): (A) PPNT without plasma treatment; (B) PPNT after being activated for 12 s by non-thermal plasma generated by diffuse coplanar surface barrier discharge.

2 MATERIALS AND METHODS

2.1 Mites

The mites used in this study were: *Acarus siro*, *Dermatophagoides pteronyssinus*, *D. farinae* and *Tyrophagus putrescentiae*. The species originated from laboratory cultures kept at the Crop Research Institute in Prague. The *Dermatophagoides* strains were originally obtained from the Food and Environmental Research Agency (Sand Hutton, York, UK) (*D. pteronyssinus*) and from the Department of General and Molecular Biology of the Medical University of Silesia (Katowice, Poland) (*D. farinae*). *Acarus siro* and *Tyrophagus putrescentiae* specimens were originally collected from Bustehrad, Czech Republic, in April 1996. The mites were mass reared in plastic tissue cell chambers with filter cap plugs (IWAKI, Prague). *A. siro* and *T. putrescentiae* specimens were reared on a mixture of oat flakes, wheat germ and Pangamin (dried yeast extract) (Rapeto, Bezdruzice, Czech Republic) (10:10:1). The mites were mass reared as described previously.¹⁹ *Dermatophagoides* specimens were reared on diet composed of dog food (Ontario-pet; Placek, Podebrady, Czech Republic), wheat germ, Aqua-tropic (dried fish food) (LonBio; Aqua Tropic Lounsky, Praha, Czech Republic), Pangamin and gelatin (Serva Electrophoresis GmbH, Heidelberg, Germany) (10:10:3:1). Before the experiment, the mites were collected with a camel hair brush from the surface of the tissue chambers under a Stemi 2000 C dissection microscope (Carl Zeiss, Jena, Germany). Unsexed adults were used for the biotest.

2.2 Chitosan/metal textiles

Spunbond polypropylene non-woven textile (PPNT) of 50 g m⁻² (Pegas Nonwovens, Czech Republic) was coated using a 2 wt% solution of medium-molecular-weight chitosan, with claimed 75–85% deacetylation (Sigma-Aldrich, St Louis, MO), in 2% (v/v) acetic acid (Sigma-Aldrich). PPNT was chosen because of its wide utilisation in the packing and furnishing industry. Prior to the coating procedure, samples of PPNT were activated by atmospheric air plasma treatment in order to improve their inherently poor wettability by water-based chitosan gel. The role of plasma treatment is to create a sufficient amount of polar functional groups on the surface of the PPNT fibres. Non-thermal plasma was generated by diffuse coplanar surface barrier discharge (DCSBD) under the following operating conditions: atmospheric air pressure; $f = 15$ kHz; $P = 400$ W; $U = 9.5$ kV_{rms}; treatment time 12 s. The apparatus for the DCSBD plasma treatment was described previously by Rahel *et al.*²⁰ The improved wettability of plasma-activated PPNT samples is apparent from Fig. 1.

Immediately after the plasma treatment, PPNT samples were immersed in the chitosan gel and placed into the waterbath shaker

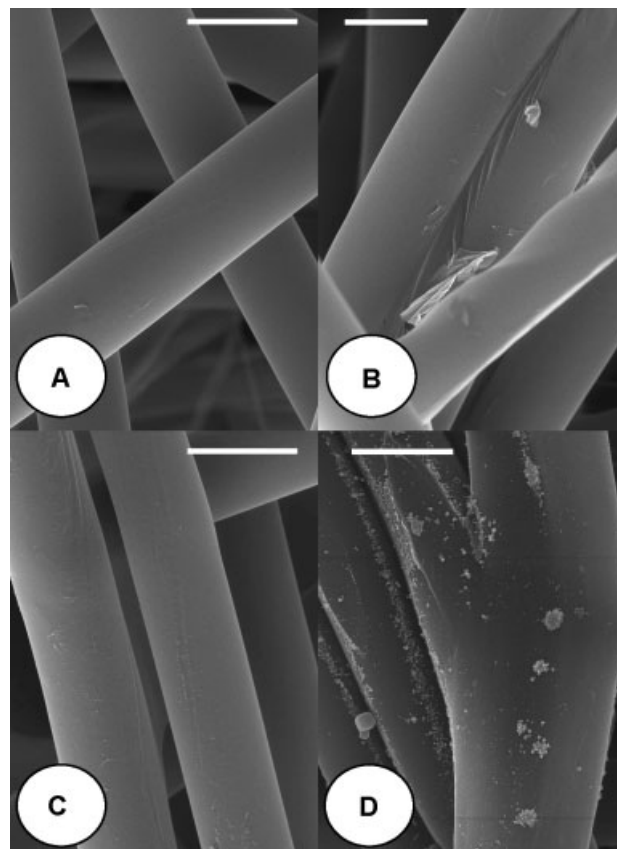


Figure 2. Morphology changes of investigated PPNT fibres under a scanning electron microscope: (A) initial spunbond polypropylene non-woven textile (PPNT) without any treatment; (B) chitosan coating on PPNT; (C) Ag-loaded chitosan coating; (D) crystals of Ag₂O on chitosan-coated fibres.

for 1 h at 60 °C. After drying at room temperature, the remaining traces of acetic acid were neutralised by dipping in 25% ammonia and washed vigorously with deionised water to remove products of neutralisation and weakly attached chitosan homopolymer. A water wash was conducted until no change in pH was observed. The average mass of the chitosan coating prepared in this way was 0.22 ± 0.05 g g⁻¹ PPNT. SEM micrographs of the samples revealed that the chitosan coating completely enveloped all individual fibres within the PPNT volume (Fig. 2). A TESCAN VEGA II SBH scanning electron microscope (Brno, Czech Republic) offering a resolution of 2 nm was employed. Prior to the SEM analysis, all samples were coated with gold using a magnetron-sputtering device.

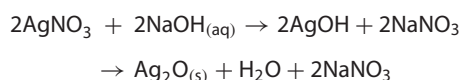
Taking advantage of the high metal ion binding capacity of chitosan,²¹ PPNT/chitosan samples were loaded with Ag⁺, Cu²⁺ and Zn²⁺ by immersing them in a water solution of 0.1 M AgNO₃ (P-Lab, Prague), 0.2 M CuSO₄ (Sigma-Aldrich) and 0.1 M ZnCl₂ (Sigma-Aldrich) respectively. The loading process was performed in light-tight bottles placed on a magnetic stirrer (300 rpm) for 24 h at room temperature. Loaded samples were left to dry at room temperature.

Quantitative analysis of Ag⁺ mass immobilised on a chitosan coating was made by potentiometric titration. Sample coating was diluted in 60 °C of 7 M HNO₃ (Sigma-Aldrich) and titrated with NaCl (Sigma-Aldrich) in the absence of light. The change in the potentiometric curve was monitored using an Orion 4-Star Benchtop pH/ISE meter (Thermo Fisher Scientific) equipped with

a Perfect IONTM combination Ag/S electrode (Mettler Toledo). The procedure resulted in $282 \pm 10 \text{ mg Ag}^+ \text{ g}^{-1}$ chitosan. This corresponds to $62 \pm 16 \text{ mg Ag}^+ \text{ g}^{-1}$ PPNT. The mass of Cu^{2+} and Zn^{2+} is expected to be of the same order of magnitude. A recently published paper on silver immobilisation on polypropylene (PP) fibre activated by an atmospheric pressure plasma treatment²² states that only $3.8 \pm 0.1 \text{ mg g}^{-1}$ Ag/PP can be immobilised on a PP surface without chitosan coating under comparable conditions. This clearly demonstrates the benefit of employing a chitosan coating as an effective binder for metal ions.

An experiment addressing the issue of PPNT/chitosan/ Ag^+ stability in water was made by immersing PPNT/chitosan/Ag samples in 1 M NaOH (Kittfort, technical grade) for 10 s. This treatment converts chitosan-adhered silver compounds of metallic Ag and AgNO_3 into water-insoluble Ag_2O , giving samples of PPNT/chitosan/ Ag_2O coating.²²

Testing of the wash-off stability of the initial PPNT/chitosan/ Ag^+ coating revealed its solubility in distilled water. This issue would, of course, hamper the future practical utilisation of the coating. Wash-off testing was done by repetitive ultrasonication in 60 °C distilled water for 30 min per cycle. Three wash-off cycles of PPNT/chitosan/ Ag^+ resulted in a tenfold reduction in initial Ag concentration to $6 \pm 2 \text{ mg g}^{-1}$ Ag/PPNT. Ten wash cycles resulted in a reduction in Ag beyond the detection limit. Analysis suggested that the phenomenon observed may have been due to the presence of a significant amount of AgNO_3 precipitate on the chitosan film.²² If this is true, then dipping in concentrated NaOH would be expected to convert adhered AgNO_3 into Ag_2O via the following reaction steps:



Three wash-off cycles of PPNT/chitosan/ Ag_2O resulted in only a twofold reduction in the initial concentration of immobilised silver to $26 \pm 8 \text{ mg g}^{-1}$ Ag/PPNT. Further wash-off cycles did not change this concentration level in any way. This result confirms that NaOH dipping has the beneficial effect of stabilising the functional coating against the wash-off effect. The conversion to Ag_2O is manifested by the sample turning a strong dark-brown colour.

To evaluate the toxic effect of immobilised Ag compounds alone, i.e. without the presence of a chitosan binding film, samples of PPNT loaded with weakly attached AgNO_3 and Ag_2O were prepared. Plasma-activated PPNT was immersed in 0.2 M AgNO_3 (P-Lab, Prague) for 1 h at room temperature and removed without squeezing out the excess liquid absorbed inside the fabric. Drying at room temperature forced dissolved Ag^+ and NO_3^- ions to precipitate on the surface of PPNT fibres in the form of weakly attached AgNO_3 microcrystals. A concentration of 0.2 M was chosen with respect to the relative mass of liquid absorbed inside PPNT (approximately 5 g g^{-1} PPNT) in order to obtain a final concentration of Ag precipitate similar to that of PPNT/chitosan/ Ag^+ , i.e. 100 mg g^{-1} Ag/PPNT. Samples of PPNT/ Ag_2O were prepared by immersing PPNT/ AgNO_3 in 1 M NaOH (Kittfort, technical grade) for 10 s, followed by a gentle distilled water wash to remove excess NaOH.

2.3 Biotest design

The experiments were carried out in 10 mL weighing bottles of 25 mm diameter (cat. number 2602; Vitrum, Prague).¹⁴ One circle of filter paper (10 mm diameter) and the tested textile were added

into every bottle. The moisture was controlled by adding 100 μL of distilled water. The bottle was not ventilated. Into every bottle, ten unsexed adults were added in ten replicates per type of textile and species. The bottles were kept in an incubator at 25 °C in the dark for 24 h. The numbers of dead mites were then counted under a dissection microscope. Any individual not exhibiting movement under a physical stimulus was considered to be dead. The same experimental design was used for the control treatments, where applications used distilled water only.

The effect of variously treated textile on the mortality of mites was tested by the chi-square test with multiple comparisons of proportions using Statistics 9 software (Analytical Software, Tallahassee, FL). The analyses were used to compare the effect of textile treatment (i.e. PPNT, PPNT/chitosan, PPNT/chitosan/ Ag^+ , PPNT/chitosan/ Cu^{2+} , PPNT/chitosan/ Zn^{2+}), the toxicity of Ag^+ (PPNT, PPNT/ AgNO_3 , PPNT/ Ag_2O), and the effect of silver derivatisation (PPNT/chitosan/ Ag^+ and washed PPNT/chitosan/ Ag_2O).

3 RESULTS

3.1 The effect of various chitosan/metal finishing on the mortality of mites

In biotests, the mortality of mites was up to 15% in the PPNT (control). The mortality of mites on textile treated with chitosan (PPNT/chitosan) did not differ from the control, with the exception of *D. farinae*. The PPNT/chitosan/ Ag^+ treated textile increased the mortality of all species of tested mites at least to 80% in biotests, while the addition of PPNT/chitosan/ Zn^{2+} and PPNT/chitosan/ Cu^{2+} did not lead to any significant differences in mortality between the treated textile and PPNT or PPNT/chitosan in *T. putrescentiae*. In *A. siro*, the mortality increased in PPNT/chitosan/ Cu^{2+} and PPNT/chitosan/ Zn^{2+} treated textile in comparison with PPNT up to 78 and 42% respectively. In *D. pteronyssinus*, significantly higher mortality was found on PPNT/chitosan/ Cu^{2+} than on PPNT; the mortality increased to 30% on PPNT/chitosan/ Cu^{2+} . In *D. farinae*, the mortality on PPNT/chitosan and PPNT/chitosan/ Cu^{2+} treated textile was higher than on PPNT, increasing up to 38 and 31% respectively. As Ag-loaded chitosan showed the best acarocidal effect, further research was focused on this particular metal.

3.2 The effect of silver on the mortality of mites

The mortality of mites depended on the type of silver loading into textile and species of mites (Fig. 3B). The chi-square test showed differences among types of impregnated textile in all species tested, i.e. *A. siro* ($\chi^2_{(3,396)} = 96$, $P < 0.001$), *D. farinae* ($\chi^2_{(3,396)} = 28$, $P < 0.001$), *D. pteronyssinus* ($\chi^2_{(3,396)} = 28$, $P < 0.001$) and *T. putrescentiae* ($\chi^2_{(3,396)} = 98$, $P < 0.001$). PPNT loaded with silver alone, i.e. without the chitosan coating, had a considerable effect on the mortality of mites too. PPNT/ Ag_2O samples showed a lower acarocidal effect than PPNT/ AgNO_3 . While PPNT/ AgNO_3 had a greater toxic effect than PPNT/chitosan/ Ag^+ or PPNT/chitosan/ Ag_2O for *Dermatophagoides* species, it had a similar toxic effect on mortality for *A. siro* and *T. putrescentiae*. When the mortality on PPNT/chitosan/ Ag^+ and PPNT/chitosan/ Ag_2O was compared, there were no significant differences in mite species, with the exception of *T. putrescentiae*. In *T. putrescentiae*, conversion to water-insoluble Ag_2O decreased the mortality from 86 to 64%.

In general it may be concluded that investigated mites are susceptible to Ag_2O silver salt as well, which is of great practical

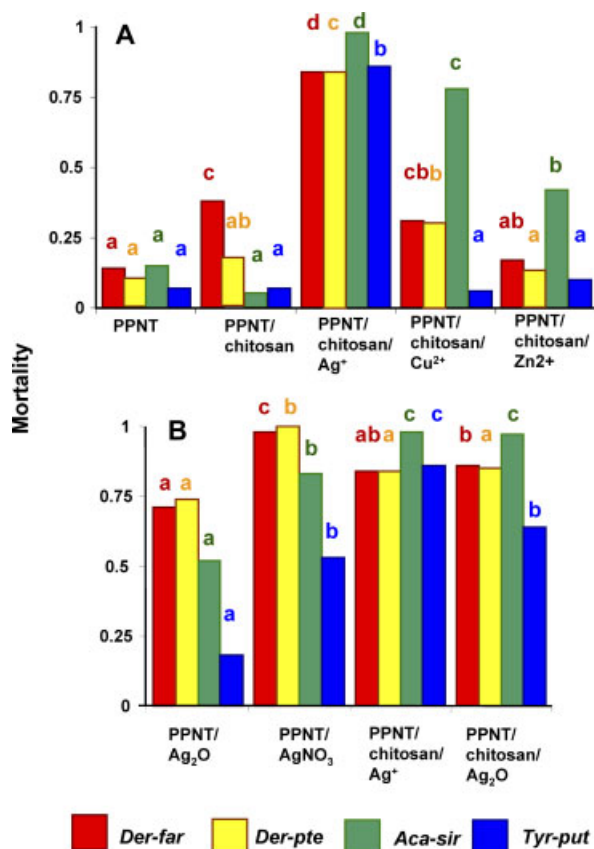


Figure 3. The mortality of tested species of mites in biotests on variously treated textiles: (A) comparison of treated textiles; (B) comparison of the mortality on textile and Ag₂O- and AgNO₃-treated textile; the letters above the columns indicate the significant differences among the treatments in the species, based on multiple comparisons for proportions at the $\alpha = 0.05$ level. PPNT: initial spunbond polypropylene non-woven textile without any treatment (control); PPNT/chitosan: chitosan coating on PPNT; PPNT/chitosan/metal: metal-loaded chitosan coating; PPNT/Ag₂O: PPNT loaded with weakly attached Ag₂O; PPNT/AgNO₃: PPNT loaded with weakly attached AgNO₃; Aca-sir: *Acarus siro*; Der-pte: *Dermatophagoides pteronyssinus*; Der-far: *D. farinae*; Tyr-put: *Tyrophagus putrescentiae*.

importance. This experiment with chitosan-free samples gave conclusive evidence for Ag atoms playing a primary role in the acaricidal action of chitosan/Ag structures.

4 DISCUSSION

The present study showed that plasma-treated fibres with chitosan/Ag⁺ coating are highly toxic to synanthropic mites (*Acarus siro*, *Dermatophagoides pteronyssinus*, *D. farinae* and *Tyrophagus putrescentiae*), while PPNT/chitosan/Zn²⁺ and PPNT/chitosan/Cu²⁺ were negligibly effective with respect to the mortality of mites. In the present study, LD₅₀ values were not estimated because different chitosan/metal concentrations were not tested owing to the experimental design. However, observed mortality on PPNT/chitosan/Ag⁺ ranged from 84 to 98%. Such toxicity of chitosan/Ag⁺ is comparable with that of conventionally used acaricides (benzylbenzoate, permethrin/pyriproxyfen/benzylbenzoate, permethrin/S-bioallethrin/piperonylbutoxide and neem).¹⁴

The toxicity of chitosan alone cannot explain the high level of acaricidal activity observed. In previous observations, the addition of chitosan to rearing diets accelerated the population growth of

T. putrescentiae in comparison with the rearing diet (Hubert J, unpublished results). Toxicity was found for both Ag₂O and AgNO₃. PPNT/Ag₂O samples showed a lower acarocidal effect than PPNT/AgNO₃. The mortality reduction can be explained by the loss of some weakly adhering silver nitrate precipitates in the NaOH dip during the Ag₂O conversion process. The conclusion is that metal ions, especially Ag⁺ ions, are responsible for toxicity. The primary role of chitosan is to increase the absolute concentration of permanently immobilised silver compounds on the chemically inert surface of PPNT fibres. Conversion of loaded silver to Ag₂O contributes to the coating stability towards water wash, without sacrificing its acaricidal activity.

The mode of action is not clear. There are three hypothetical modes: toxicity abrasion, oral toxicity and toxicity through the effect on associated bacteria. The abrasion of mites is well documented: abrasive materials (i.e. diatomaceous earths) destroy the epicuticle of mites, leading to their desiccation and death.²³ The Ag⁺ is known for its bactericidal activity, which increases slightly after Ag⁺ loading into chitosan.⁹ The studied mites developed a bacterial community,^{24,25} including parasitic endosymbionts of high 16S RNA sequences similar to *Candidatus Cardinium herzegi*.²⁶ The mites can be dependent on microbe cohabitation, as documented for some arthropods,²⁷ and the suppressive effect is through regulation of the associated microbial community. However, the mode of action of the materials tested in this study is unknown.

The selected model species are well documented as allergen producers; there are 35 characterised biochemical allergens within the tested species (www.allergen.org). The main habitats for *Dermatophagoides pteronyssinus* and *D. farinae* are house dust, carpets and mattresses;¹² for example, mites were found in 47% (from 353) house dust samples in Poland.²⁸ *Tyrophagus putrescentiae* is present in the same habitats as previous species,²⁹ but also in stored food,³⁰ dried ham³¹ and pet feeds.³² *Acarus siro* mainly occurs in cheeses and cereal food for human consumption.³⁰ In stored grain, 27 and 15% of grain samples were infested by *A. siro* and *T. putrescentiae* respectively.³³

The observed acaricidal effect of PPNT/chitosan/Ag⁺ indicates two possible major applications of chitosan/Ag⁺: (i) in antiallergenic textile mattresses and carpets; (ii) to cover polypropylene foils in the packaging of food or feed products. The eggs and hypoppy of mites are metabolically low-activity stadia with high resistance to every type of control, and eggs comprised a very substantial part of the *Dermatophagoides* spp. population in mattresses.¹²

Chitosan/Ag⁺ textiles are stable, and the protection effect on the mites should be longer than for chemical pesticides. In the present study, the washing of textile with chitosan/Ag⁺ and chitosan/Ag₂O functional coatings has been simulated. For the chitosan/Ag₂O system, a stable level of immobilised silver (and hence toxicity to mites) within a range of ten washing cycles has been shown.

The mites contaminate stored products during whole food/feed processing, even in the kitchen.³⁴ Infestations of mites are mostly low in number,^{30,33} usually below the detection limit of screening techniques.³⁵ Such a low infestation can penetrate to packed food or feed and can result in massive infestations later. Recently, it was demonstrated in laboratory experiments that the dried fruit mite *Carpoglyphus lactis* (Linnaeus, 1758) was able to penetrate from an infested into an uninfested package of dried apricots.³⁶ European Union legislation dictates a food and feed 'zero level of mite infestation' in cereals, dried fruit and feed, but the restrictions

are far from realistic. The improvement in packaging as a result of using chitosan/Ag⁺ can increase the protection of packaged food or feed from mite infestation. However, the data obtained here are from laboratory experiments, and, for the two applications suggested, field experiments will be necessary. Through the application of chitosan/Ag⁺ it will be possible to establish that chitosan/Ag⁺ fibres have great potential as a means to control the mite population in homes via novel antiallergenic materials and/or pest protective food packaging.

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