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FUNGICIDE EFFICACY OF NANOFIBRE TEXTILES CONTAINING CHEMICAL PRESERVATIVES FOR PROTECTION OF WOODEN MATERIALS – PRELIMINARY STUDY

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ABSTRACT

The aim of presented work was to verify a possible application of nanofibre polyvinyl alcohol (PVA) textiles doped with commercially available biocides for chemical preservation of wooden materials. Fungicide efficacy of biocides based on Propiconazole, Tebuconazole, IPBC was evaluated on Norway spruce samples (*Picea abies* Karst. L.) 25 x 25 x 4 mm covered by the nanofibre textiles, against the wood destroying fungus *Coniophora puteana* during 28 days. Decay resistance of spruce samples with the fungicidally treated layer was valued on the base of weight loss (Δ_m) criteria. The durability of spruce samples significantly increased after incorporation of fungicides into the nanofibre textiles (Δ_m from 0.2 to 3.7 %), but the influence of the stabilization process was also reflected.

KEYWORDS: Wood protection, nanofibre textiles, *Coniophora puteana*, fungicides, electrospinning.

INTRODUCTION

The use of wood is limited by its susceptibility to biotic organisms that may damage its structure and deteriorate its properties. Wood-rotting fungi reduce several strength properties of wood before significant weight loss is detected, that may have a devastating effect on stability of construction. The service life of wooden products is usually increased by using of more durable wood species or by chemical treatment of wood. Nowadays, where environmental or health legislation has forced restrictions on usage of some chemicals, commonly used to protect timber, there is an urgent need to investigate new alternative fungicides with no impact to surrounding environment and human health.

Promising method for a new functional way to protect wood could be also nanotechnology. A lot of research has been recently concerned to verify application of preparations based on nanoparticles of metal (silver, zinc, copper, titan, etc.) in regard to fungal growth prevention (Matsunaga et al. 2009; Kartal et al. 2009; Shah et al. 2010; Bak et al. 2012; De Filpo et al. 2013; Kűnniger et al. 2014). Nanobiocides have the potential to affect the field of wood preservation through the creation of new and unique metal biocides with improved properties (Kartal et al. 2009). Nanometal preparations have high dispersion stability and low viscosity allowing for more uniform particulate distribution (Clausen 2007) and location of preparations nanocomponents in wood tissue structure (Ważny and Kundzewicz 2008).

Another approach in application of nanotechnology for wood protection is additional application of nanofibre textile augmented by antimicrobial agents. Nanofibre textile, prepared by electrospinning, is a special material characterized by an enormous surface to volume ratio, flexibility in surface functionalities, high porosity (Esfandarani and Johari 2010; Prosecká et al. 2012) and it is possible to apply the nanofibre coating directly on the surface of the protected materials. The textiles can be produced of various polymers, based on required properties. Their properties may be further enhanced and modified by the addition of active substances.These properties predetermined the use of nanofibres in various applications. Nanofibre materials are broadly used in medicine as antimicrobial protection for tissue engineering (wound healing, drug delivery, scaffolds) (Cui et al. 2010; Parizek et al. 2012).

Melaiey et al. (2005) used silver (I)-imidazole cyclophane gem-diol complexes encapsulated by electrospun nanofibres. The resulting nanofibre mats were found to be effective against *Staphylococcus aureus* and comparable to 0.5 % $AgNO_3$. The fiber mats also showed antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *S. aureus, Candida albicans, Aspergillus niger*, and *Saccharomyces cervisiae* (Melaiye et al. 2005). Elzatahry et al. (2012) explored the antimicrobial and antifungal activities of nanofibres produced by electrospinning of N-heterocyclic carbene gold(I) complexes with PVA. Nanofibres containing the gold (I) chloride complex exhibit localized activity against both of the Gram-positive (*S. aureus* and *Bacillus subtilis*) and one of the Gram-negative (*Micrococcus leuteus*) strains tested.

The experiments with use of nanotextiles enhanced by metal nanoparticles in the field of civil engineering were also carried out (Rácová et al. 2013a, b; Ryparová et al. 2013). Results showed that antimicrobial effect of PVA-based nanofibre textiles with incorporated ions of silver and copper gave antifungal character against filamentous fungi, wood-destroying fungi and algae occurring in the buildings to the textiles.

The present work was focused on utilization of nanofibre textile doped with active substances commonly contained in commercially available fungicides (based on Propiconazole, Tebuconazole, IPBC) against the growth and damaging activity of the wood destroying fungi *Coniophora puteana*.

MATERIAL AND METHODS

Fungicides

The organic fungicides Preventol A8, Wocosen TK 50, Wocosen TK 20 (provided by Stachema, CZ) were used in concentration of 2 % in polymer solution (Tab.1). Organic active substances belong to the 1,2,4-triazoles (Propiconazole, Tebuconazole) and carbamates (IPBC). These fungicides are light-stable, non-volatile, better resist leaching by water and therefore suitable for long-term protection.

Nanofibre textiles

The basic polymer solution for electrospinning was prepared by dissolving 375 g 16 % PVA Solviol (Fichema, CZ), 3 g 85 % Phosphoric acid (P-lab, CZ), 4.4 g Glyoxal (Merck, USA) in 117 g of distilled water. For fungal assay the biocide nanofibre textiles were prepared by blending the active substance in the polymer solution prior to electrospinning.

The electrospinning equipment was a Nanospider TMLB 500 device (Elmarco, CZ) in the Center for Nanotechnology at the Czech Technical University in Prague. Electrospinning parameters were optimized at ambient conditions as follows; laboratory temperature (25°C), relative humidity (35 %), the applied voltage (79.5 kV), the distance between electrodes (140 mm). The nanofibres were spun on a polypropylene base substrate (spunbond) with the width 500 mm, weight of 18 g.m^{-2} and an antistatic treatment.

Due to the high dissolvability of the PVA membrane in contact with water it is appropriate to stabilize the nanofibre textile. In our experiment we used 3 common ways of stabilization (Yeom and Lee 1996, Ding et al. 2002, Franco et al. 2012). Membrane stabilization was done by heat treatment and chemical crosslinking by methanol and combination of acetone/glutaraldehyde to compare the influence of stabilization to antifungal efficiency of textiles. For the heat stabilization, PVA textiles were put into the oven set at 140°C during 30 minutes. Chemical crosslinking was done by immersing the nanotextiles in absolute methanol and acetone with GA (200 ml acetone, 4 ml GA; pH 2-3) for 4 hours.

Tab. 1: Wood preservatives and their active substances used in experiment.

Solution mark	Fungicide	Active substance
A	Wocosen 20 TK	10 % **Propiconazole** and 10 % **IPBC**(3-iodo-2-propynyl-N-butylcarbamate)
B	Wocosen 50 TK	50 % **Propiconazole**(± cis/trans(1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1,2,4-triazole)
C	Preventol A8 + Wocosesn 20 TK	3 g **Tebuconazole**(α-[2-(4-Chlorophenyl)ethyl]-α-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol) + 20g Wocosen 50 TK

Tests of antifungal activity:

The antifungal activity tests of the fungicidally treated nanofibre textiles were made by means of:

- screening tests,
- modified mycological test by EN 113 (1997)

Screening efficacy test of treated nanofibre textiles

The antifungal efficacy of each tested solution contained in nanofibre textile against the wood-destroying fungus *Coniophora puteana* (Schumacher ex Freis) Karsten was determined in 3 Petri dishes (total 27 dishes). The circlular samples of nanofibre textiles (φ = 30 mm) were placed into the central point of each Petri dish on malt agar culture medium and then in a distance of 20 mm from the border of textile were deposit 4 fungal inocula. Screening test was running at the incubating temperature of 23± 1°C, during 14 days. Evaluation of the wood decaying fungus growth was done after 7 and 14 days. The slowdown in the growth of fungi mycelia around the nanofibre textiles circle (i.e., formation of inhibition zones) and also intensity of the potential growth of mycelia on the textile surface was monitored (Fig. 1).

Test of decay resistance of spruce samples with layer from treated nanofibre textiles

The anti-decay resistance of the spruce specimens covered by nanofibre textiles doped by biocides was searched against *Coniophora puteana* (Schumacher ex Freis) Karsten, which is well-known as common indoor basidiomycetes, by which the brown decay in wood is caused. Fungal decay test was based on the EN 113 (1997) with some modifications as follows: - Using of specimens with a smaller dimension of 25 x 25 x 4 mm (L x R x T) instead of 50 x 25 x 15 mm (L x R x T); - treatment of specimens by application of chemically treated nanotextile surface layer instead of their impregnation; - shorter time of fungal test only 4 weeks instead of 16 weeks; - exposure of specimens in Petri dishes with a diameter of 100 mm instead of in a 1-liter Kolle's flasks.

Mycological tests were performed under sterile conditions in thermostat at temperature of 23± 1°C. Two spruce wood (*Picea abies* Karst L.) samples (1 reference = sample covered by PVA textile without fungicides; 1 treated = sample covered by PVA doped by fungicides), all with known initial mass (m_0), were placed to every Petri dish with enlarging mycelium of testing fungus on carrying support from glass tubes to ensure that surfaces of samples were not in direct contact with malt agar medium.

After 4 weeks, the specimens were pulled out from Petri dishes; the mycelia were carefully brushed from the surface of spruce samples. The anti-decay resistance tests were valued on the basis of specimen's weight losses in percentage by the Eq.:

$$\Delta m = \frac{m_0 - m_F}{m_0} \times 100 \qquad (\%)$$

where : m_F - mass of the decayed sample in the oven dry state,
m_0 - mass of the sound sample before decay in the oven dry state.

RESULTS AND DISCUSION

Screening test

In the first stage of the experiment, the possibility of fungicidal efficiency of chemically treated nanofibre textiles was performed. The experiment showed (Tab. 2, Fig. 1) that nanofiber textile with addition of fungicides have antimycotic properties. All applied fungicides inhibited the growth of *C. puteana* mycelia due to their diffusion into the surrounding malt agar media. Otherwise, it was confirmed that the pure PVA textiles have no biocidal effect on fungus *C. puteana*.

Tab. 2: Screenings of the antifungal efficacy of fungicidally treated nanofibre textiles against the wood destroying fungus after 14 day test.

Tested fungicide	***Coniophora puteana* after 14 days**					
	Heat		**Methanol**		**Acetone/GA**	
	Inhibition zone (mm)	Growth on papers (mm)	Inhibition zone (mm)	Growth on papers (mm)	Inhibition zone (mm)	Growth on papers (mm)
Solution A	1-7	0-2	2-11	0	5-14	0
Solution B	5-12	0	10-15	0	12-18	0
Solution C	3-9	0-1	6-13	0	9-15	0
Reference PVA	0	30	0	30	0	26
Control	0	30	0	30	0	30

Note:
Each value in the table corresponds to the arithmetic mean of three nanofibre textile samples situated in three Petri dishes.

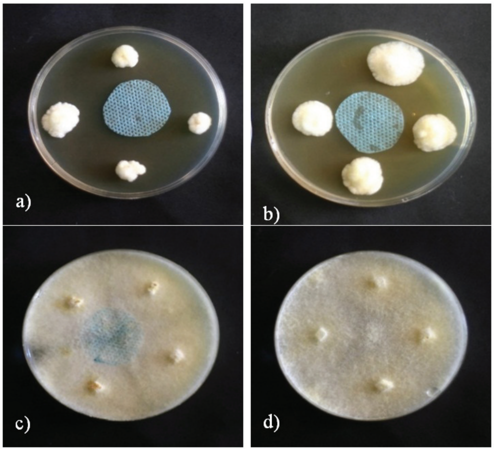

Fig. 1: Screening test of fungicide efficacy of wood preservatives incorporated in nanofibre textiles crosslinked by methanol after 14 days: a) sample with addition of solution B, b) sample with addition of solution A, c) reference sample with pure PVA textile d) control sample without nanofibre textile.

Mycological test

Weight losses of treated wood after 4 weeks exposure to *C. puteana* are presented in Fig. 1. Results of experiment with nanofibre textiles doped by fungicides showed that wood samples with fungicidally treated nanofibre textiles had significantly lower weight losses than nontreated reference samples. The efficiency of IPBC, Propiconazole and Tebuconazole fungicides was substantially equal (Fig. 2, Tab. 3). As table indicates, nanofibre textile with Wocosen 50 TK was the relatively most efficient and Wocosen 20 TK had the poorest biocidal effect. This can be explained by a presence of IPBC in the fungicide solution, which is mainly used against moulds and staining fungi growth. Activity of IPBC against wood-destroying fungi can be increased by higher concentration or enhanced with borates (Reinprecht 2008).

Tab. 3: Weightlosses (Δ_m) of the spruce samples covered by fungicidally treated nanofibre textiles after 4 weeks.

Stabilization	**Weight loss (%)**							
	Sol. A		**Sol. B**		**Sol. C**		**Ref**	
	$\bar{x}$	**SD**	$\bar{x}$	**SD**	$\bar{x}$	**SD**	$\bar{x}$	**SD**
Methanol	0.68	0.21	0.41	0.49	0.54	0.75	7.63	1.93
Acetone/GA	0.47	0.10	0.20	0.13	0.43	0.46	3.75	1.58
Heat	3.73	0.60	3.51	3.06	3.66	1.77	10.19	3.85
Untreated wood	-	-	-	-	-	-	10,72	1,95

Note

$\bar{x}$ Arithmetic mean value, SD – Standard deviation

Totally 60 samples of the Norway spruce wood were attacked by the fungus *C. puteana*.

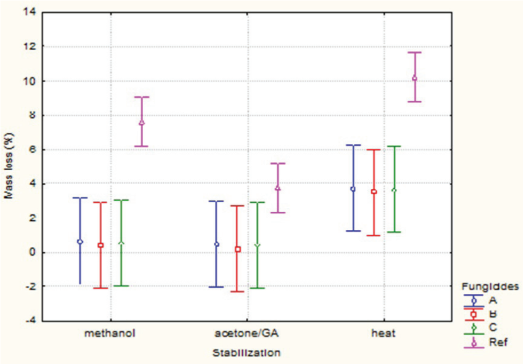

Fig. 2: Weightlosses "Δ_m" of spruce samples after their decay with the brown-rot fungus Coniophora puteana.

On the other side, the decay resistance of the covered spruce samples by nanofibrous textiles against brown-rot fungus was significantly influenced by method of crosslinking the textiles. Wooden samples overlaid with nanotextiles crosslinked by chemical way had higher resistance against the wood destroying fungus in comparison with the nanotextile samples stabilized by heat. Chemically crosslinked nanotextiles by methanol achieved weight loss between 0.03 to 0.96 % and by acetone/GA from 0.01 to 0.59 %. Weight losses of heat stabilized nanotextiles varied from 0.14 to 6.12 %. So, it can be hypothesized that during the heat treatment the stability of active substance could probably be impaired, because producer guarantees stability up to 60°C.

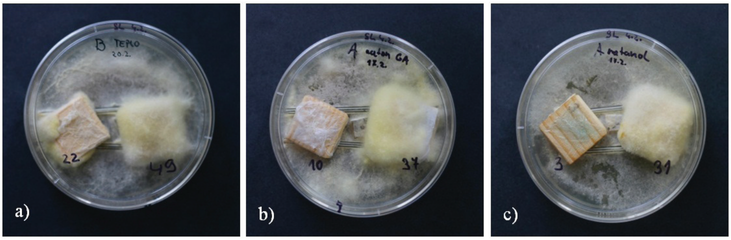

Fig. 3: Mycological test of fungicide efficacy of nanofibre textile after 4 weeks a) stabilized by heat, b) crosslinked by acetone/GA, c) crosslinked by methanol.

Even the differences among reference samples were evident. Pure PVA textiles without fungicides substance should not have any biocidal effect against fungi. However, in our work sprucesamples overlaid with reference nanotextile crosslinked by acetone/GA generally performed better (Tab. 3). Their average mass losses varied between 2.25 and 6.57 % after attack by *Coniophora puteana*. On the other side, decay resistance of samples overlaid with nanotextile crosslinked by methanol (Δ_m from 5.2 to 11.34 %) and with nanotextile stabilized by heat (4.52 to 13.95 %) was evidently lower. This could be explained by the presence of glutaraldehyde in crosslinking solution. Glutaraldehyde is one of the most effective crosslinking agent (Bolto et al. 2009) but also has some biocide properties. As was reported by Yusuf (1996) glutaraladehyde was superior to wood formalization by non- or low-formaldehyde reagents in enhancing the resistance of treated wood against decay in comparison with DMDHEU or glyoxal treatment.

CONCLUSIONS

The application possibility of polymer nanofibre textiles augmented by addition of commercially available biocides agents for wood protection against *Coniophora puteana* was studied in this work. The experiment confirmed fungicidal efficiency of selected agents in the form of nanofibre textiles as a carrier network. Results obtained in the pilot study illustrate that the decay resistance of spruce samples significantly increased due to the presence of organic fungicides incorporated in nanofibre textiles. But on the other side, it was proved, that the efficiency of polymer textiles depends on the method of crosslinking.

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REFERENCES

1. Bak, M., Yimmou, B.M., Csupor, K., Nemeth, R., Csoka, L., 2012: Enhancing the durability of wood against wood destroying fungi using Nano-Zink. In: International Scientific Conference on Sustainable Development & Ecological Footprint. March 26-27, 2012. Sopron, Hungary, 6 pp.
2. Bolto, B., Tran, T., Hoang, M., Xie, Z.L., 2009: Crosslinked poly(vinyl alcohol) membranes. Progr. Polym. Sci. 34(9): 969-981.
3. Clausen, C.A., 2007: Nanotechnology: Implications for the wood preservation industry. IRG/WP/07-30415. Stockholm, Sweden: International Research Group on Wood Protection. 13 pp.
4. Cui, W., Zhou, Y., Chang, J., 2010: Electropsun nanofibrous materials for tissue engineering and drug delivery. Science and Technology of advanced Materials 11(1): 11 pp.

5. De Filpo, G., Palermo, A.M., Rachiele, F., Nicoletta, F.P., 2013: Preventing fungal growth in wood by titanium dioxide nanoparticles. International Biodeterioration & Biodegradation 85: 217-222.
6. Ding, B., Kim, H.Y., Lee, S.C., Lee, D.R., Choi, K.J., 2002: Preparation and characterizationof nanoscaled poly(vinyl alcohol) fibersvia electrospinning. Fibers and Polymers. 3(2): 73-79.
7. Elzatahry, A.A., Al-Enizi, A.M., Elsayed, E.A., Butorac, R.R., Al-Deyab, S. S., Wadaan, M.A.M., Cowley, A.H., 2012: Nanofiber composites containing N-heterocyclic carbine complexes with antimicrobial activity. International Journal of Nanomedicine 7: 2829-2832.
8. EN 113, 1979: Wood preservatives—method of test for determining the protective effectiveness against wood destroying Basidiomycetes— determination of the toxic values.
9. Esfandarani, M.S., Johari, M.S., 2010: Producing porous nanofibres. In: Proceedings of the 2nd international conference NANOCON 2010. Pp 1-6, Olomouc, Czech Republic.
10. Franco, R.A., Min, Y.K., Yang, H.M., Lee, B.Y., 2012: On stabilization of PVPA/ PVAelectrospun nanofiber membrane and its effect on material properties and biocompatibility. Journal of Nanomaterials, Article ID 393042, 9 pp.
11. Kartal, S.N., Green, F.III., Clausen, C.A., 2009: Do the unique properties of nanometals affect leachability or efficacy against fungi and termites? International biodeterioration & biodegradation 63(4): 490-495.
12. Künniger, T., Gerecke, A.C., Ulrich, A., Huch, A., Vonbank, R., Heeb, M., Wichser, A., Haag, R., Kunz, P., Faller, M., 2014: Release and environmental impact of silver nanoparticles and conventional organic biocides from coated wooden façades. Environmental Pollution 184: 464-471
13. Matsunaga, H., Kiguchi, M., Evans, P., 2008: Microdistribution of copper-carbonate and iron oxide nanoparticles in treated wood. Journal of Nanoparticle Research 11(5): 1087-1098.
14. Melaiye, A., Sun, Z., Hindi, K., Milsted, A., Ely, D., Reneker, D.H., Tessier, C.A., Youngs, W.J., 2005: Silver (I)-imidazole cyclophane gem-diol complexes encapsulated by electrospun tecophilic nanofibers: Formation of nanosilver particles and antimicrobial activity. Journal of the American Chemical Society 127(7): 2285-2291.
15. Parizek, M., Douglas, T., Novotna, K., Kromka, A., Brady, M.A., Renzing, A., Voss, E., Jarosova, M., Palatinus, L., Tesarek, P., Ryparova, P., Lisa, V., Santos, A.M., Bacakova, L., 2012: Nanofibrous poly(lactide-co-glycolide) membranes loaded with diamond nanoparticles as promising substrates for bone tissue engineering. International Journal of Nanomedicine 7: 1931-1951.
16. Prosecká, E., Buzgo, M., Rampichová, M., Kocourek, T., Kochová, P., Vysloužilová, L., Tvrdík, D., Jelínek, M., Lukáš, D., Amler, E., 2012: Thin-layer hydroxyapatite deposition on a nanofiber surface stimulates mesenchymal stem cell proliferation and their differentiation into osteoblasts. Journal of Biomedicine and Biotechnology, Article ID 428503, 10 pp.
17. Rácová, Z., Wasserbauer, R., Rypárová, P., 2013a: Microscopic filamentous fungi in buildings, preventing their occurrence and their remediation using nanofibers. Advanced Materials Research 649: 89-92.
18. Rácová, Z., Ryparová, P., Wasserbauer, R., Nežerka, V., Tesárek, P., Demo, P., 2013b: Verification of the use of PVA based nanofiber textiles as rehabilitation for the structures attack by wood decaying fungi. In: 5th International Conference NANOCON 2013 Conference Proceedings. Ostrava: TANGER, spol.s.r.o.

19. Reinprecht, L., 2008: Wood protection. (Ochrana dreva). Handbook, Technical University in Zvolen - Slovakia, 453 pp (in Slovak).
20. Ryparová, P., Wasserbauer, R., Rácová, Z., Demo, P., Tesárek, P., 2013: Algicidal properties nanofibrous fabrics doped by metals. In: 5th International Conference NANOCON 2013 Conference Proceedings. Ostrava: TANGER, spol.s r.o., ISBN 978-80-87294-44-4.
21. Shah, V., Dobiasova, P., Baldrian, P., Nerud, F., Kumar, A., Seal, S., 2010: Influence of iron and copper nanoparticle powder on the production of lignocellulose degrading enzymes in the fungus *Trametes versicolor*. Journal of Hazardous Materials 178(1-3): 1141-1145.
22. Wažny, J., Kundzewicz, A., 2008: Conditions and possibility of nanobiocides formulation for wood protection. The International Research Group on Wood Protection, (Istanbul – Turkey), IRG/WP 08-30467, 9 pp.
23. Yeom, C.K., Lee, K.H., 1996: Pervaporation separation of water-acetic acid mixtures throughpoly (vinyl alcohol) membranes crosslinked with glutaraldehyde. Journal of Membrane Science 109(2): 257-265.
24. Yusuf, S., 1996: Properties enhancement of wood by cross-linking formation and its application to the reconstituted wood products. Wood research: Bulletin of the Wood Research Institute Kyoto University 83: 140-210.

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