ACTA FACULTATIS XYLOLOGIAE ZVOLEN, 58(1): 51−58, 2016
Zvolen, Technická univerzita vo Zvolene
DOI: 10.17423/afx.2016.58.1.06

DECAY INHIBITION OF MAPLE WOOD WITH NANO-ZINC OXIDE USED IN COMBINATION WITH ESSENTIAL OILS

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ABSTRACT

This work investigates the anti-decay efficiency of nano-zinc oxide (ZnO-*Nano*: 0.1, 0.33, 1 or 3% ethanol dispersions), three essential oils (clove, oregano and thyme: 1 or 3% ethanol solutions), as well as their mixtures – introduced into samples 25 × 25 × 3 mm (L × R × T) of maple wood (*Acer pseudoplatanus*) by dipping at 20°C/1hour. The anti-decay efficiency of all treating substances was valued against the white-rot fungus *Trametes versicolor*, based on a drop of mass losses of treated samples in relation to untreated ones during their 6 week's exposure in fungal mycelia in petri dishes. The best decay inhibition of maple wood was achieved after its treatment with the 3% nano-zinc oxide (retention of ca 5 $kg \cdot m^{-3}$) when the mass losses of wood decreased from 20.4% (untreated wood) to 4.5% (treated wood). On contrary, the anti-decay efficiency of essential oils against the *T. versicolor* was either poor (using clove and thyme oils) or even none (using oregano oil). Individual essential oils did not have synergetic / promoting effect for increasing the anti-decay efficacy of nano-zinc oxide.

Key words: maple wood, nano-zinc oxide, essential oils, decay.

INTRODUCTION

In present the nanotechnologies are applied for wood protection, as well. Bioactive nano-compounds with dimensions of 10 to 100 nm have specific physical and biological properties. However, their transport to wood is limited by: (1) potential closure of lumens in vessels by tyloses, (2) dimension of openings in cell walls, and (3) dimension of micro-pores in cell walls (FREEMAN & MCLNTYRE 2008, KARTAL *et al.* 2009). Antimicrobial properties of several nano-metals are good thanks not only their chemical composition, but also to their small dimensions, charge and dispersive properties (MCCRANK 2009, CLAUSEN *et al.* 2010).

From the point of view of wood protection, the nanotechnologies are the most important at increasing its photostability (e.g. by nano-titanium dioxide and nano-zinc oxide), resistance to fire (e.g. by nano-silicon dioxide and nano-titanium dioxide), and resistance to biological pests. Natural resistance of wood against bacteria, moulds, decaying fungi or insects can be increased by nanoparticles of silver (AKHTARI & AREFKHANI 2013, MOYA *et al.* 2014), nano-zinc oxide (CLAUSEN *et al.* 2010, BAK *et a*l. 2012, AKHTARI & AREFKHANI 2013, STANKOVIĆ *et al.* 2013, MANTANIS *et al.* 2014, REINPRECHT *et al.* 2015), nano-zinc borate (LYKIDIS *et al.* 2013, MANTANIS *et al.* 2014), nanoparticles of copper (AKHTARI & AREFKHANI 2013) and nano-copper oxide (MANTANIS *et al.* 2014), or nano-titanium dioxide (MARZBANI & MOHAMMADNIA-AFROUZI 2014), but also by other nano-metals and nano-compounds with biocide efficiencies.

For wood protection also the essential oils are prospective, because they are usually health-friendly and cause only small problems in terms of liquidation of treated products after their service-life (CHITTENDEN & SINGH 2011). However, some disadvantages at using of essential oils for wood protection can be connected with their high volatility and lower efficciency (BATISH *et al*. 2008). Coming out from results of more scientific works, cited for example by PÁNEK *et al*. 2014, the best anti-fungal efficiency have essential oils in which composition are present: (1) phenolic compounds, such as carvacrol (e.g. oregano and savory oils), thymol (e.g. thyme oil), eugenol (e.g. clove oil), and / or (2) oxygenated compounds, such as elemol and cinamaldehyde (e.g. cinnamon oil).

The aim of this work was to search anti-decay efficiency of nano-zinc oxide (ZnO-*Nano*) in combination with three types of essential oils (clove, oregano, thyme) against the white-rot fungus *Trametes versicolor*.

MATERIALS AND METHODS

Maple wood

In the experiment were used samples of maple wood (*Acer pseudoplatanus*) with a dimension of 25 × 25 × 3 mm (L/longitudinal/ × T/tangential/ × R/radial/), without biological damages, knots or other growth inhomogeneity, having a density in the oven dry state in range of 518–588–653 $kg \cdot m^{-3}$. In total 252 samples, 168 treated and 84 untreated (reference), were processed. Their sterilization before treatment with chemical substances (nano-zinc oxide, essential oils) was performed at a temperature of 103 ± 2°C/4 h. Sterilization of their surfaces before fungal attack was performed with a 30 W germicidal lamp (Chirana, Slovakia) from a distance of 1 m at a temperature of 22 ± 2°C/0.5 h.

Nano-zinc oxide

Nano-zinc oxide (ZnO-*Nano*), which particles had dimensions of 50 nm, was obtained from Sigma-Aldrich. Maple samples were treated with its 0.1, 0.33, 1 and 3% ethanol dispersions.

Essential oils

Three pure essential oils of pharmacopoeia quality were used in the experiment (Table 1). They for treatment of maple samples were used in a form of 1 and 3% ethanol solutions, or in dispersion systems when have been applied in mixtures with ZnO-*Nano*.

Tab. 1 Essential oils used in the experiment.

Common name	Scientific name	Major effective components
Clove	*Syzygium aromaticum*	Eugenol (82%), Caryophyllene (16.5%)
Oregano	*Origanum vulgare*	Carvacrol (71.8%), Thymol (5%), gamma-Terpinene (4.5%)
Thyme	*Thymus vulgaris*	Thymol (41.3%), p-cymol (22.6%), gamma-Terpinene (7.7%), Carvacrol (2.9%)
Source of essential oils: Nobilis Tilia s.r.o., Czech Republic		

Treatment methods

The samples of maple wood were dried at a temperature of 103 ± 2°C to a constant weight (m_0) determined with an accuracy of 0.001 g, and to a constant volume (V_0) determined with an accuracy of 0.01 mm for their all three dimensions. Immediately thereafter the samples were chemically treated by dipping technology (20°C, 100 kPa, 60 minutes) with ethanol solutions / dispersions of substances "A", "B" and "C":

A. ZnO-*Nano* (0.1, 0.33, 1 or 3% ethanol dispersion system),
B. essential oil (1 or 3% ethanol solution of clove, oregano or thyme oil),
C. mixture of ZnO-*Nano* & essential oil.

The treated samples were in 1 minute deprived of liquid drops using a filter paper and immediately thereafter weighted with an accuracy of 0.001 g ($m_{Treated}$). The retention of solutions / dispersions of the treating substances into wood ($R_{Solution}$) was calculated from the increased weights of maple samples after treatment ($m_{Treated} - m_0$) and from their initial volume (V_0), by the Eq. 1. The retention of the treating substances into wood (R), at mixtures separately for ZnO-*Nano* and essential oil, was calculated from the value $R_{Solution}$ and the mass percentage concentration of the treating substance (c [%]) used in the individual treatment "A" or "B", respectively in the mixture treatment "C", by the Eq. 2.

$$R_{Solution} = \frac{m_{Treated} - m_0}{V_0} \ [\text{kg·m}^{-3}] \quad (1)$$

$$R = R_{Solution} \frac{c}{100} \ [\text{kg·m}^{-3}] \quad (2)$$

Wood decaying fungus

The white-rot fungus *Trametes versicolor* (Linnaeus ex Fries) Pilat, strain CTB 863 A was used for a decay attack of reference (un-treaded) and treated samples.

Fungal attack

The fungal attack of the reference and chemically treated maple samples with the white-rot fungus *Trametes versicolor* was performed according to a modified standard EN 113 (1996), with these changes: - smaller dimension of samples 25 × 25 × 3 mm instead of 50 × 25 × 15 mm; - other treatment process (dipping instead of vacuum impregnation); - shorter time of decay (6 weeks instead of 16 weeks).

Intensity of the fungal attacks in the treated and reference maple samples was evaluated on the basis of their mass losses (Δm). For these aims, the samples before and after fungal attack were similarly air-conditioned at a temperature of 20 ± 1°C and relative humidity of 60 ± 2% to a constant weight, determined with an accuracy of 0.001 g. The mass losses of the treated samples were determined from their weights in conditioned states before ($m_{Treated}$) and after ($m_{Fungal\text{-}Attack}$) fungal attack, by the Eq. 3.

$$\Delta m = \frac{m_{Treated} - m_{Fungal-Attack}}{m_{Treated}} \cdot 100 \ [\%] \quad (3)$$

RESULTS AND DISCUSSION

Retention of treating substances

The retentions of the treating solutions / dispersions, and retentions of the active treating substances, i.e. the zinc-oxide and essential oils, into maple samples are present in Table 2. The retentions of the active substances into the maple wood continually increased with their higher concentration in the treating systems. For example, retentions of ZnO-Nano increased from ca 0.16–0.17 to ca 4.5–5.5 kg·m^{-3} with increasing of its concentration from 0.1 to 3%.

Tab. 2 Retentions of treating substances into samples of maple wood with known densities.

TREATING SUBSTANCE	WOOD DENSITY ρ_0 [kg·m^{-3}] $\bar{x}$ (SD)	RETENTION [kg·m^{-3}]		
		$R_{Solution}$ $\bar{x}$ (SD)	$R_{ZnO\text{-}Nano}$ $\bar{x}$	$R_{Essential\ oil}$ $\bar{x}$
0.1 % ZnO-*Nano*	592 (0.01)	170 (0.02)	0.17	–
0.33 % ZnO-*Nano*	581 (0.04)	167 (0.02)	0.55	–
1 % ZnO-*Nano*	585 (0,02)	153 (0.02)	1.53	–
3 % ZnO-*Nano*	567 (0.02)	187 (0.01)	5.62	–
1 % Clove	587 (0.04)	165 (0.02)	–	1.65
3 % Clove	584 (0.02)	175 (0.02)	–	5.25
0.1 % ZnO-*Nano* + 1 % Clove	585 (0.01)	168 (0.02)	0.168	1.68
0.33 % ZnO-*Nano* + 1 % Clove	574 (0.01)	184 (0.01)	0.607	1,84
1 % ZnO-*Nano* + 1 % Clove	581 (0.02)	144 (0.01)	1.440	1.44
3 % ZnO-*Nano* + 1 % Clove	586 (0.02)	181 (0.02)	5.430	1.81
0.1 % ZnO-*Nano* + 3 % Clove	588 (0.01)	149 (0.02)	0.149	4.47
0.33 % ZnO-*Nano* + 3 % Clove	586 (0.02)	154 (0.03)	0.509	4.63
1 % ZnO-*Nano* + 3 % Clove	576 (0.01)	139 (0.01)	1.390	4.17
3 % ZnO-*Nano* + 3 % Clove	580 (0.03)	162 (0.01)	4.860	4.86
1 % Oregano	597 (0.02)	152 (0.01)	–	1.52
3 % Oregano	603 (0.02)	184 (0.02)	–	5.53
0.1 % ZnO-*Nano* + 1 % Oregano	631 (0.00)	156 (0.01)	0.156	1.56
0.33 % ZnO-*Nano* + 1 % Oregano	591 (0.03)	142 (0.01)	0.468	1.42
1 % ZnO-*Nano* + 1 % Oregano	621 (0.02)	137 (0.02)	1.368	1.37
3 % ZnO-*Nano* + 1 % Oregano	598 (0.01)	155 (0.01)	4.650	1.55
0.1 % ZnO-*Nano* + 3 % Oregano	617 (0.01)	142 (0.01)	0.142	4.27
0.33 % ZnO-*Nano* + 3 % Oregano	640 (0.02)	140 (0.01)	0.463	4.21
1 % ZnO-*Nano* + 3 % Oregano	629 (0.01)	145 (0.02)	1.447	4.34
3 % ZnO-*Nano* + 3 % Oregano	629 (0.01)	149 (0.02)	4.463	4.46
1 % Thyme	569 (0.02)	146 (0.02)	–	1.46
3 % Thyme	603 (0.02)	150 (0.02)	–	4.50
0.1 % ZnO-*Nano* + 1 % Thyme	591 (0.01)	177 (0.02)	0.177	1.77
0.33 % ZnO-*Nano* + 1 % Thyme	607 (0.02)	153 (0.02)	0.506	1.53
1 % ZnO-*Nano* + 1 % Thyme	594 (0.02)	168 (0.01)	1.680	1.68
3 % ZnO-*Nano* + 1 % Thyme	584 (0.01)	169 (0.01)	5.060	1.69
0.1 % ZnO-*Nano* + 3 % Thyme	584 (0.02)	172 (0.02)	0.172	5.15
0.33 % ZnO-*Nano* + 3 % Thyme	596 (0.01)	163 (0.02)	0.538	4.89
1 % ZnO-*Nano* + 3 % Thyme	585 (0.01)	163 (0.02)	1.628	4.88
3 % ZnO-*Nano* + 3 % Thyme	627 (0.02)	169 (0.01)	5.070	5.07

NOTE: $\bar{x}$ = mean value; SD = standard deviation is in the parentheses; n = mean values are from 4 samples of one type.

Efficiency of nano-zinc oxide against white rot

Nano-zinc oxide (ZnO-*Nano*) significantly improved the anti-decay resistance of the maple wood at its exposition to the white-rot fungus *Trametes versicolor* (Table 3, Figures 1 and 2). Mass loss of the reference maple wood was 20.4%. At increased concentrations of ZnO-*Nano* from 0.1 to 3% (retentions into samples increased from ca 0.165 to ca 5.0 kg·m^{-3}), the white-rot fungus *T. versicolor* in maple wood caused lower mass losses from 13.8% (at using 0.1% ZnO-*Nano*) to 4.5% (at using 3% ZnO-*Nano*). The tendency of a higher anti-decay efficiency of nano-zinc oxide with its increased concentration (or amount in wood – Table 2) is shown by linear correlations, with coefficients of determination R^2 from 0.42 to 0.56 (Figures 1 and 2). A significant anti-decay influence of the concentration of nano-zinc oxide (c [%]) and thus also its retention into wood (R [kg·m^{-3}]) searched by correlation analyses as well as REZAZADEH *et al.* (2014).

Similar results related to a good anti-decay efficiency of nano-zinc oxide against white-rot fungi achieved as well as KARTAL *et al.* (2009) CLAUSEN *et al.* (2010), AKHTARI & AREFKHANI (2013), MANTANIS *et al.* (2014), REZAZADEH *et al.* (2014), and REINPRECHT *et al.* (2015).

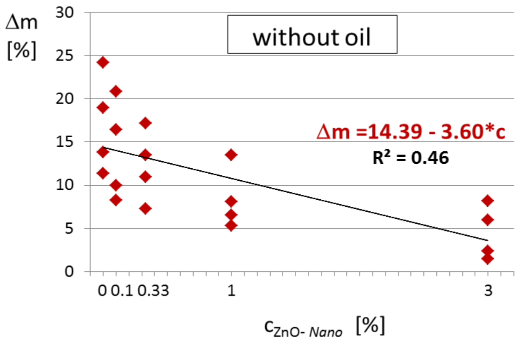


Fig. 1 Linear correlation between: (1) the concentrations (c) of ZnO-*Nano* used for treatment of maple samples, and (2) the mass losses (Δm) of treated samples caused by the white-rot fungus *Trametes versicolor*.

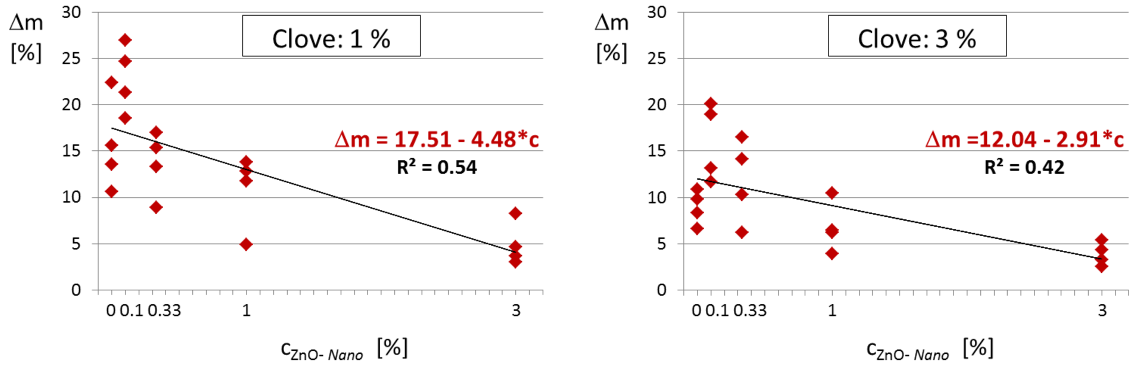
Tab. 3 Mass losses (Δm) of treated and reference samples 25 × 25 × 3 mm of maple wood (*Acer pseudoplatanus*) due to 6-week's action of the white-rot fungus *Trametes versicolor.*

TREATING SUBSTANCE	Δm – MASS LOSS [%]			
	WITHOUT OIL	ESSENTIAL OIL		
	$\bar{x}$ (SD)	Clove $\bar{x}$ (SD)	Oregano $\bar{x}$ (SD)	Thyme $\bar{x}$ (SD)
Reference – untreated maple wood	20.4 (11.7)	–	–	–
0.1 % ZnO-*Nano*	13.8 (5.8)	–	–	–
0.33 % ZnO-*Nano*	12.2 (4.2)	–	–	–
1 % ZnO-*Nano*	8.4 (4.1)	–	–	–
3 % ZnO-*Nano*	4.5 (3.1)	–	–	–
1 % Essential oil	–	15.5 (5.0)	27.8 (2.4)	16.7 (3.9)
3 % Essential oil	–	8.9 (1.8)	19.9 (2.5)	13.9 (3.5)
0.1 % ZnO-*Nano* + 1 % Essential oil	–	22.9 (3.7)	21.8 (4.3)	15.8 (4.9)
0.33 % ZnO-*Nano* + 1 % Essential oil	–	13.6 (3.5)	10.1 (4.0	11.6 (3.5)
1 % ZnO-*Nano* + 1 % Essential oil	–	10.8 (4.1)	7.9 (3.4)	7.7 (3.1)
3 % ZnO-*Nano* + 1 % Essential oil	–	4.9 (2.3)	3.6 (1.5)	4.4 (2.2)
0.1 % ZnO-*Nano* + 3 % Essential oil	–	16.0 (4.2)	19.8 (3.1)	13.5 (1.0)
0.33 % ZnO-*Nano* + 3 % Essential oil	–	11.8 (4.5)	8.5 (3.2)	9.2 (4.9)
1 % ZnO-*Nano* + 3 % Essential oil	–	6.8 (2.7)	8.0 (4.2)	5.8 (3.5)
3 % ZnO-*Nano* + 3 % Essential oil	–	3.9 (1.2)	5.4 (1.1)	3.9 (2.4)

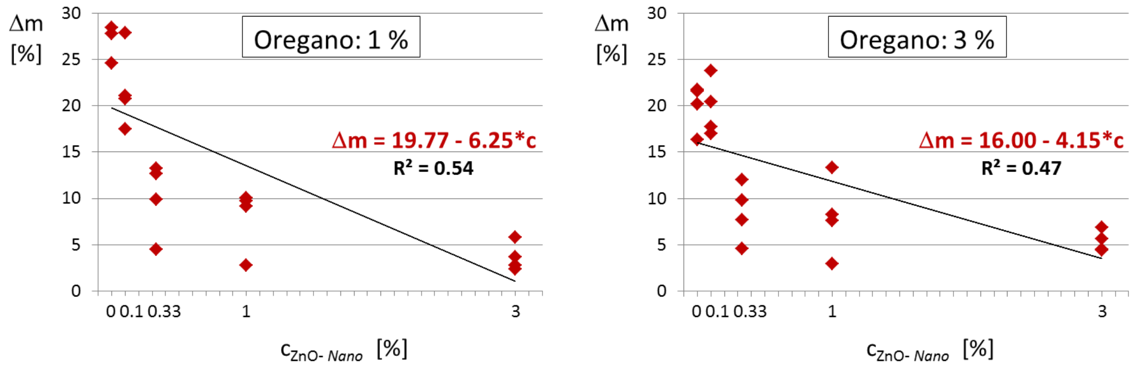
$\bar{x}$ = mean value; SD = standard deviation is in the parentheses; n = mean values are from 4 modified samples of one type, or from 84 reference samples.

Efficiency of essential oils against white rot

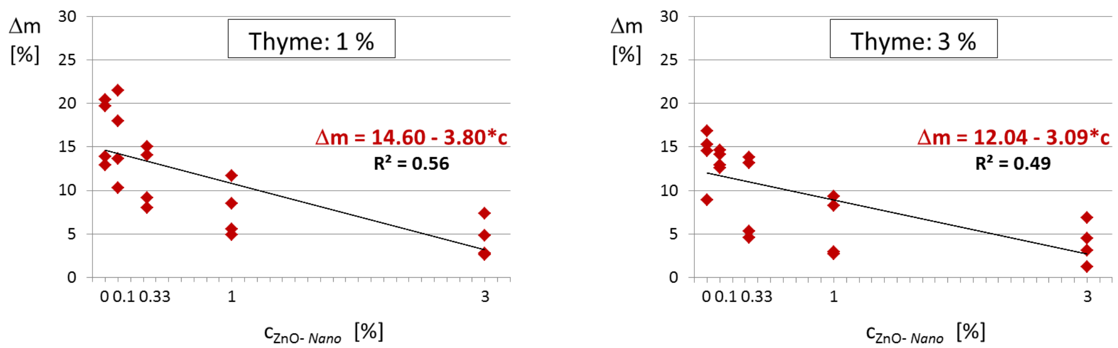
Clove and thyme oils partly increased the anti-decay resistance of the natural maple wood, when in their presence the mass losses of wood decreased from 20.4% to 9–17%. However, oregano oil had none anti-decay efficiency, when mass losses of wood treated with this oil were higher or comparable (from 20 to 28%) with mass losses of reference maple wood (20.4%) (Table 3).



a) ZnO-*Nano* used in mixture with clove oil: ZnO-*Nano* & Clove



b) ZnO-*Nano* used in mixture with oregano oil: ZnO-*Nano* & Oregano



c) ZnO-*Nano* used in mixture with thyme oil: ZnO-*Nano* & Thyme

Fig. 2 Linear correlation between: (1) the concentrations (c) of ZnO-*Nano* used for treatment of maple samples in mixtures with essential oils (clove, oregano, thyme), and (2) the mass losses (Δm) of treated samples caused by the white-rot fungus *Trametes versicolor.*

In the work of PÁNEK *et al*. (2014), at examination of the anti-decay efficiency of ten essential oils against the white-rot fungus *Trametes versicolor* and the brown-rot fungus *Coniophora puteana*, there was found result that the *T. versicolor* is evidently less sensitive to several essential oils than the *C. puteana*. A lower sensitivity of white-rot fungi to essential oils determined VODA *et al.* (2003), as well. The results of BAYRAMOGLU & ARICA (2009) showed a very high production of the extracellular ligninolytic enzyme laccase by the *T. versicolor*, at which this enzyme destroys lignin in wood and continuously deactivates phenolic compounds with the highest anti-fungal effects present as well as in essential oils, such as thymol, carvacrol, and eugenol.

Efficiency of nano-zinc oxide in mixtures with essential oils against white rot

The results of experiments did not show any positive synergetic – it means promoting – effect of essential oils for the increase of nano-zinc oxide efficiency against the white-rot fungus *Trameses versicolor* (Table 3, Figures 1 and 2). This result is in accordance with our previous work (REINPRECHT *et al.* 2015) when acrylic resin decreased fungicidal effect of nano-zinc oxide against the fungi *T. versicolor* and *C. puteana*.

Lower values of the positive parameter "a", and also lower values of the negative parameter "b", there in the linear relations "$\Delta m = a + b \times c$", where "c" is the concentration of nano-zinc oxide, were obtained for the highest 3% concentration of essential oils in treating mixtures. It can be explained by a certain (less important) anti-decay efficiency of used essential oils themselves, it means also at 0% concentration of ZnO-*Nano* (Fig. 2).

CONCLUSSIONS

- Nano-zinc oxide (ZnO-*Nano*) was confirmed as a biologically affective substance in suppressing white rot, at testing its efficiency against the fungus *Trametes versicolor*.
- Anti-fungal efficacy of nano-zinc oxide increased with its concentration from 0.1 to 3%, i.e. with its higher retention into maple wood.
- Essential oils – clove, oregano and thyme – applied in 1 and 3% concentrations had only minimum anti-decay effect against the white-rot fungus *T. versicolor*. This result is in accordance with knowledge of other works that white-rot fungi can degrade not only wooden lignin but also phenolic compounds present as well as in essential oils, such as thymol, carvacrol, and eugenol.
- Individual essential oils applied in mixtures with nano-zinc oxide did not have synergetic / promoting effect for increase the anti-decay efficacy of this nano-compound.

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Acknowledgement

This work was supported by the Slovak Research and Development Agency under the contract No. APVV-0200-12.

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