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THE EVALUATION OF *IN VITRO* FUNGITOXICITY LEVEL OF CHOSEN PHENOLIC COMPOUNDS NATURALLY EXISTING IN WOOD BY USING THE AG NUTRIENT AGAR MEDIUM TESTS*

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Abstract. This paper presents the process and the results of studies on fungitoxicity level of the chosen phenolic compounds occurring in wood that can be responsible for the natural resistance of this material to decay by fungi. All together 14 substances were examined: Eugenol, Vanillic acid, Isoeugenol, Cyclohexanone, Resorcinol, Syringaldehyde, 2,6-dimethoxyphenol (Syringol), Pyrogallol, 4-methoxybenzoic acid (Anisiic acid), 2--furaldehyde, Furanone, 4-allyl-2,6-dimethoxyphenol, Tetramethyl-4-butanediamine and 3',5'-dimethoxyacetophenone and 6 different mixtures of these compounds imitating their natural coexistance in wood. For the tests the modified AG nutrient agar medium method based on using a series of media plates of various saturations of the tested substance was used. *Trametes versicolor* and *Laetiporus sulphureus* served as the test fungi. All together 20 variants of the experiment were pursued on 2400 plates. On this basis the fungitoxicity level of each substance was determined and they were evaluated in regards of their potential usefulness in the practical protection of trees and wood.

Key words: nutrient tests, *Laetiporus sulphureus*, *Trametes versicolor*, natural protection of wood, phenolic compounds

INTRODUCTION

The phenolic compounds occurring in wood are, in the opinion of many specialists, one of the main factors responsible for the natural resistance of wood against decay by fungi [Charlwood and Rhodes 1990, Davin et al. 1992, Evensen et al. 2000, Kermasha et al. 1995, Obst 1998, Theander and Lundgren 1989, Wallace and Fry 1994]. The statistical analyses consisting of confrontation of the test results for the scope of prefe-

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rences and the trophic abilities of the selected fungi species in regards to wood decay of the individual tree species and the amount of the known phenolic compounds occurring in their wood, proved the existence of the potential substances from this group that can be the natural inhibitors of the mycelial growth [Zarzyński 2009]. Such as: 3',5'--dimethoxyacetophenone and 2-methoxy-4-(propenyl**)-**phenol (Isoeugenol), also: 2--cyclopentene-1-one-2-hydroxy-3-methylo; Furanone (2-furanone); 1,4-butanodiamine-2,3-dimethoxy N,N,N',N' tetramethyl; Resorcinol (1,3-dihydroxybenzene); 1,6- anhydro-beta-D-glucopyranose (Levoglukosan); Acetylbenzoic-2,5-dimethoxy acid; 2,5--furandion-3-methylo and 2,6-dimethoxyphenol (Syringol). The subject of this research were the nutrient medium tests of the selected phenolic substances occurring in wood pursued under laboratory conditions using AG method. Their purpose was to evaluate the fungitoxicity level of those compounds in order to determine the possibility of their potential usefulness in the practical protection both of the living trees and the wood material against fungal decay.

MATERIALS AND METHODS

In order to estimate the potential usefulness of the chosen phenolic compounds identified in wood for its protection against fungal decay the AG method was used [Grzywacz 1987]. It is a modified way of assessing the antifungal properties of the individual substances by the nutrient agar medium tests. In its favour speaks a comparatively easy performance, universality and high degree of precision. The method is based on the use of series of the artificial substrates with an increasing content of the potential antifungal substance and the measurement of the mycelium inoculated on them in order to compare its growth rate in reference to the control mycelium. Agar-wort medium served as the substrate (25 ml of wort ('Jablonowo Brewery' – the pasteurized wort from a single production batch and with the same chemical parameters was used), 75 ml of water, 2 g of agar). The medium was first sterilized by autoclaving in temperature of 121°C. Then, after cooling it down to the temperature of 50°C, the relevant amount of the solution or the fungicide suspension was added to it and such a preparated mixture was poured into the previously sterilised 90 mm diameter Petri plates, in the amount of 10 ml per plate. To track precisely the mechanism of the mycelium reaction to the specified chemical substance 11 different concentrations of it were used: 0.1 ppm, 0.5 ppm, 1 ppm, 5 ppm, 10 ppm, 50 ppm, 100 ppm, 500 ppm, 1000 ppm, 5000 ppm and 10 000 ppm and the control variant – without any chemical substance additive (pure medium). Each combination was repeated five times. After the medium had solidified, the mycelium of the test fungi of the dimensions 5 mm × 5 mm was inoculated in the centre of the plate.

The mycelium was measured either when on any of the plates it achieved the size of 90 mm, that is, it reached the brims, or after seven days. The mycelium colony (diameter) was measured twice and in a criss-cross way at an angle of 90° and afterwards the results of those measurements were averaged. By comparing the diameter of the mycelium grown on the plates containing the consecutive concentrations of the fungicide with the diameter of the mycelium obtained on the control plates, the range that included the ED_{50} coefficient (Medium Effective Dose – it is the concentration of the active substance required to stop the mycelium growth by exactly 50% in comparison to the mycelium on the control plates) was determined for each substance. On this basis

the fungitoxicity class was determined for each examined substance in reference to the specified species of the test fungus. For that purpose the scale presented in the Table 1 [Grzywacz 1987] was used.

Table 1. The proposal classes of fungicides fungitoxicity specified by nutrient agar medium tests [Grzywacz 1987]

Tabela 1. Propozycja klas fungitoksyczności środków grzybobójczych określonych metodą pożywkową [Grzywacz 1987]

The class of fungitoxicity Klasa fungitoksyczności	The toxicity evaluation of the fungicide against particular species of testing fungi Ocena toksyczności preparatu w stosunku do określonego gatunku grzyba testowego	The ED_{50} value, ppm Wartość ED_{50}, ppm
1	extraordinarily toxic niezwykle toksyczny	< 0.1
2	very toxic bardzo toksyczny	0.1-1
3	toxic toksyczny	1.1-10
4	medium-toxic średnio toksyczny	11-100
5	little toxic mało toksyczny	101-1000
6	very little toxic bardzo mało toksyczny	1001-10 000
7	non-toxic nietoksyczny	> 10 000

Two fungi were used as the testing species: Turkeytail (*Trametes versicolor* (L.) Lloyd) and Sulphur Shelf (*Laetiporus sulphureus* (Bull.) Murrill). They cause two different types of wood rot (*T. versicolor* – white rot, homogenous, *L. sulphureus* – brown rot) and they are the standard species used is the research of such type.

20 different variants of the experiment were pursued. 14 compounds of phenol characteristics naturally occurring in wood and 6 of their mixtures imitating their natural coexistance in wood were subjected to the test based on the above described method.

The respective test variants were as follows:

Variant I – Eugenol
Variant II – Vanillic acid
Variant III – Isoeugenol
Variant IV – Cyclohexanone
Variant V – Resorcinol
Variant VI – Syringaldehyde
Variant VII – 2,6-dimethoxyphenol
Variant VIII – Pyrogallol
Variant IX – 4-methoxybenzoic acid
Variant X – 2-furaldehyde
Variant XI – Furanone

Variant XII – 4-allyl-2,6-dimethoxyphenol
Variant XIII – N',N',N',N'-tetramethyl-4-butanediamine
Variant XIV – 3',5'-dimethoxyacetophenone
Variant XV – Eugenol + Isoeugenol (1:1)
Variant XVI – Pyrogallol + Resorcinol (1:1)
Variant XVII – Eugenol + Resorcinol (1:1)
Variant XVIII – Eugenol + Pyrogallol (1 :1)
Variant XIX – Isoeugenol + Resorcinol (1:1)
Variant XX – Eugenol + Isoeugenol + 4-methoxybenzoic acid (1:1:1).

In order to determine the importance of the differences in the growth rate of the mycelium on the media with the increasing concentration of the investigated substances, statistical analysis with the aid of StatGraphics programme were made. ANOVA (analysis of variance) and the multiple comparison test (LSD – least-significant difference test) were used for this purpose. Separate analyses were conducted for each of the tested substances for both species of the test fungi.

RESULTS

The results of the above experiment in form of averaged diameters of mycelium grown on media containing individual concentrations of tested phenolic compounds or their mixtures for individual species of test fungi are presented in Tables 2 and 3. They contain marked ranges of concentrations that, for individual tested compounds or their mixtures, the value of ED_{50} indicator most probably falls into. The average classes of toxicity defined on that basis for individual substances (tested on two species of fungi) are presented on Figure 1. The results of the tests for significance of differences in the speed of mycelium of the test fungi species growth for the chosen substances versus their concentration in the media are presented on Figure 2-6.

In case of media tests conducted on *Laetiporus sulphureus* mycelium an average score of 5.35 on a 7 grade scale was obtained for the tested substances and their mixtures. The most effective substances include Eugenol (4) and Isoeugenol (4). In case of Eugenol it is not ruled out that its real fungitoxicity towards *L. sulphureus* is higher, as the speed of growth of fungi colony on a medium containing concentration of the substance equal 50 ppm did not statistically differ from the speed of growth observed on a media with Eugenol concentration equal 10 ppm (Fig. 2). Nevertheless, it does not change the classification of the substance to the 4 class of fungitoxicity. Whereas in case of Isoeugenol the statistical analysis showed significant differences between both boundary values (Fig. 3). On the other hand for the media test conducted on *Trametes versicolor mycelium* the average score obtained for tested substances and their mixtures was 5.55. The most effective substances included Eugenol (4th class of fungitoxicity). Similarly to the tests on *L. sulphureus* mycelium no significant statistical differences between the speed of growth of mycelium colony on a medium with concentration of the tested substance of 50 ppm and 10 ppm were observed. Therefore, it is not ruled out that the real fungitoxicity of the substance towards *T. versicolor* is higher (Fig. 2; yet without change of fungitoxicity class). For Isoeugenol the statistical analysis demonstrated occurrence of significant differences for both boundary values (Fig. 3).

Table 2. Results of nutrient agar medium tests specified by AG methods (testing fungus – *Laetiporus sulphureus* – grey color indicates the concentrations adjoining with the Medium Effective Dose for different chemical compound)

Tabela 2. Wyniki testów pożywkowych metodą AG (grzyb testujący *Laetiporus sulphureus* – szarym kolorem oznaczono stężenia, między którymi przypada dla danego środka chemicznego wartość Medium Effective Dose)

Variant of experiment (see in text) Wariant doświadczenia (patrz w tekście)	Medium diameter of mycelium colony [mm] with the concentration of [ppm] Przeciętna średnica kolonii grzybni [mm] przy stężeniu [ppm]												Class of fungitoxicity Klasa fungitoksyczności
	control kontrola	0.1	0.5	1	5	10	50	100	500	1 000	5 000	10 000	
I	39.2	38.0	37.6	37.4	34.8	34.4	**28.6**	**1.6**	0	0	0	0	**4**
II	49.2	47.6	47.4	45.6	45.2	44.0	42.2	38.8	38.2	**27.8**	**16.2**	16.0	**6**
III	42.6	41.6	41.2	41.0	40.0	32.2	**24.2**	**10.6**	0	0	0	0	**4**
IV	42.6	41.0	40.4	40.0	39.2	39.0	38.6	38.4	38.0	37.4	35.0	23.6	**6**
V	38.2	37.4	36.4	36.2	35.6	35.4	35.2	30.8	**28.6**	**18.4**	0	0	**5**
VI	39.8	39.4	38.2	37.8	37.0	36.8	36.4	36.2	35.8	**32.2**	**4.0**	3.0	**6**
VII	42.0	41.4	41.0	40.8	40.0	39.0	38.8	37.6	37.4	37.2	**35.6**	**0**	**6**
VIII	40.8	40.6	39.0	38.6	38.4	38.0	33.2	**29.2**	**12.6**	3.0	2.0	0	**5**
IX	32.4	31.2	30.8	30.0	29.8	29.4	27.2	23.8	**19.8**	**5.0**	5.0	5.0	**5**
X	41.6	39.4	39.0	36.4	36.0	35.2	34.6	33.2	**31.2**	**20.4**	12.4	0	**5**
XI	53.2	51.2	50.6	49.6	49.4	47.6	47.4	41.6	**35.2**	**22.0**	0	0	**5**
XII	59.0	53.4	53.0	51.2	46.6	46.0	31.4	**29.4**	**0**	0	0	0	**5**
XIII	40.4	37.6	36.8	35.0	29.8	26.4	25.0	24.4	23.0	**22.8**	**0**	0	**6**
XIV	41.8	37.6	32.6	32.4	32.0	31.4	30.8	30.4	30.0	**29.4**	**5.0**	5.0	**6**
XV	40.4	40.2	39.8	39.6	39.2	38.8	38.6	**37.6**	**3.0**	0	0	0	**5**
XVI	52.6	51.8	51.2	51.0	50.2	49.6	49.0	**48.4**	**24.4**	0	0	0	**5**
XVII	49.2	48.8	48.2	47.6	47.4	47.0	46.6	46.2	41.2	**38.2**	**0**	0	**6**
XVIII	47.6	46.8	46.6	44.2	43.6	42.6	40.2	35.8	**31.2**	**18.0**	0	0	**5**
XIX	48.0	44.6	42.2	41.6	40.2	38.6	35.2	33.2	31.2	**28.2**	**20.4**	0	**6**
XX	42.4	42.0	40.2	36.6	36.0	34.0	33.6	33.0	28.8	28.2	**24.2**	**0**	**6**

Table 3. Results of nutrient agar medium tests specified by AG methods (testing fungus – *Trametes versicolor* – grey color indicates the concentrations adjoining with the Medium Effective Dose for different chemical compound)

Tabela 3. Wyniki testów pożywkowych metodą AG (grzyb testujący *Trametes versicolor* – szarym kolorem oznaczono stężenia, między którymi przypada dla danego środka chemicznego wartość Medium Effective Dose)

Variant of experiment Wariant doświadczenia	Medium diameter of mycelium colony [mm] with the concentration of [ppm] Przeciętna średnica kolonii grzybni [mm] przy stężeniu [ppm]												Class of fungitoxicity Klasa fungitoksyczności
	control kontrola	0.1	0.5	1	5	10	50	100	500	1 000	5 000	10 000	
I	69.6	66.4	65.6	64.4	63.6	57.6	**51.6**	**25.0**	0	0	0	0	**4**
II	86.2	84.2	83.0	79.2	77.6	76.0	74.8	73.8	71.6	71.0	**46.4**	**40.8**	**6**
III	70.4	70.0	67.0	66.6	65.0	63.4	58.8	**41.6**	**0**	0	0	0	**5**
IV	90.0	85.8	85.2	84.6	82.0	80.8	72.2	70.4	66.4	63.4	62.8	49.0	**6**
V	89.0	80.2	79.0	75.8	74.4	72.6	71.4	69.8	**66.0**	**34.0**	0	0	**5**
VI	64.2	61.6	59.6	59.0	57.6	57.0	55.6	54.8	54.0	**53.0**	**0**	0	**6**
VII	71.4	67.6	67.2	67.0	66.4	65.0	64.2	63.6	62.4	**60.6**	**0**	0	**6**
VIII	82.0	77.2	76.4	76.2	75.4	73.0	72.4	**69.0**	**37.8**	28.0	0	0	**5**
IX	72.4	71.2	70.8	68.2	67.6	65.2	61.8	56.0	**54.2**	**29.2**	17.0	13.8	**5**
X	77.0	75.2	72.0	70.4	68.2	67.6	64.8	61.0	**58.4**	**36.6**	5.2	0	**5**
XI	81.2	80.8	79.0	78.6	77.6	77.2	76.6	74.6	57.4	**42.0**	**0**	0	**6**
XII	81.2	79.8	73.2	72.0	69.2	68.2	52.4	**51.4**	**0**	0	0	0	**5**
XIII	65.8	65.0	63.8	62.2	61.4	60.0	59.4	58.2	47.4	**40.6**	**5.0**	5.0	**6**
XIV	83.0	76.0	73.4	70.0	69.6	67.8	52.6	51.8	50.0	**48.4**	**0**	0	**6**
XV	69.8	65.0	64.2	63.2	62.2	53.0	48.8	46.8	44.8	**44.2**	**4.0**	0	**6**
XVI	90.0	88.2	83.2	80.6	79.6	78.6	78.2	73.8	**69.6**	**37.8**	0	0	**5**
XVII	71.6	69.2	67.8	65.0	63.4	62.8	61.8	59.6	56.4	**48.8**	**34.2**	0	**6**
XVIII	68.0	65.2	61.4	58.2	51.0	48.2	46.0	45.2	43.0	40.0	**38.8**	**0**	**6**
XIX	64.8	58.8	55.6	54.8	50.2	48.2	46.8	46.2	44.4	**41.2**	**30.0**	0	**6**
XX	72.8	70.2	62.8	58.8	55.6	53.2	49.6	46.6	42.4	40.0	**39.8**	**12.6**	**6**

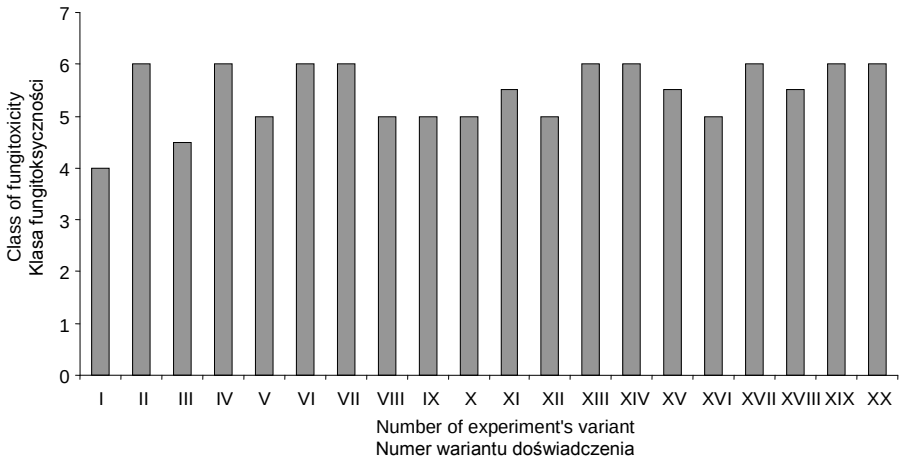

Fig. 1. Medium classes of fungitoxicity for both species of tested fungi reached by investigated phenolic compounds or their mixtures in different variants of the experiment

Rys. 1. Średnie klasy fungitoksyczności dla dwóch gatunków grzybów testowych uzyskane przez badane związki fenolowe lub ich mieszaniny w poszczególnych wariantach doświadczenia (patrz tekst)

Concentration of fungicide Stężenie fungicydu (ppm)	0	0.1	0.5	1	5	10	50	100	500	1 000	5 000	10 000
0	x											
0.1		x										
0.5			x									
1				x								
5					x							
10						x						
50							x					
100								x				
500									x			
1 000										x		
5 000											x	
10 000												x

Laetiporus sulphureus

Trametes versicolor

Fig. 2. The variance of growth range of mycelium of *Laetiporus sulphureus* and *Trametes versicolor* on media with increasing concentration of Eugenol (the grey color indicates statistically significant differences – by LSD tests at the 95% confidence level)

Rys. 2. Istotność różnic pomiędzy tempem wzrostu grzybni *Laetiporus sulphureus* i *Trametes versicolor* na pożywkach o wzrastającym stężeniu eugenolu (kolor szary wskazuje istnienie istotnej pod względem statystycznym różnicy – test NIR przy poziomie ufności 95%)

Concentration of fungicide Stężenie fungicydu (ppm)	0	0.1	0.5	1	5	10	50	100	500	1 000	5 000	10 000
0	x											
0.1		x										
0.5			x									
1				x								
5					x							
10						x						
50							x					
100								x				
500									x			
1 000										x		
5 000											x	
10 000												x

Laetiporus sulphureus

Trametes versicolor

Fig. 3. The variance of growth range of mycelium of *Laetiporus sulphureus* and *Trametes versicolor* on media with increasing concentration of Isoeugenol (the grey color indicates statistically significant differences – by LSD tests at the 95% confidence level)

Rys. 3. Istotność różnic pomiędzy tempem wzrostu grzybni *Laetiporus sulphureus* i *Trametes versicolor* na pożywkach o wzrastającym stężeniu izoeugenolu (kolor szary wskazuje istnienie istotnej pod względem statystycznym różnicy – test NIR przy poziomie ufności 95%)

Concentration of fungicide Stężenie fungicydu (ppm)	0	0.1	0.5	1	5	10	50	100	500	1 000	5 000	10 000
0	x											
0.1		x										
0.5			x									
1				x								
5					x							
10						x						
50							x					
100								x				
500									x			
1 000										x		
5 000											x	
10 000												x

Laetiporus sulphureus

Trametes versicolor

Fig. 4. The variance of growth range of mycelium of *Laetiporus sulphureus* and *Trametes versicolor* on media with increasing concentration of Pyrogalol (the grey color indicates statistically significant differences – by LSD tests at the 95% confidence level)

Rys. 4. Istotność różnic pomiędzy tempem wzrostu grzybni *Laetiporus sulphureus* i *Trametes versicolor* na pożywkach o wzrastającym stężeniu pirogalolu (kolor szary wskazuje istnienie istotnej pod względem statystycznym różnicy – test NIR przy poziomie ufności 95%)

Concentration of fungicide Stężenie fungicydu (ppm)	0	0.1	0.5	1	5	10	50	100	500	1 000	5 000	10 000
0	x											
0.1		x										
0.5			x									
1				x								
5					x							
10						x						
50							x					
100								x				
500									x			
1 000										x		
5 000											x	
10 000												x

Laetiporus sulphureus

Trametes versicolor

Fig. 5. The variance of growth range of mycelium of *Laetiporus sulphureus* and *Trametes versicolor* on media with increasing concentration of 2-furaldehyde (the grey color indicates statistically significant differences – by LSD tests at the 95% confidence level)

Rys. 5. Istotność różnic pomiędzy tempem wzrostu grzybni *Laetiporus sulphureus* i *Trametes versicolor* na pożywkach o wzrastającym stężeniu 2-furaldehydu (kolor szary wskazuje istnienie istotnej pod względem statystycznym różnicy – test NIR przy poziomie ufności 95%)

Concentration of fungicide Stężenie fungicydu (ppm)	0	0.1	0.5	1	5	10	50	100	500	1 000	5 000	10 000
0	x											
0.1		x										
0.5			x									
1				x								
5					x							
10						x						
50							x					
100								x				
500									x			
1 000										x		
5 000											x	
10 000												x

Laetiporus sulphureus

Trametes versicolor

Fig. 6. The variance of growth range of mycelium of *Laetiporus sulphureus* and *Trametes versicolor* on media with increasing concentration of Resorcinol (the grey color indicates statistically significant differences – by LSD tests at the 95% confidence level)

Rys. 6. Istotność różnic pomiędzy tempem wzrostu grzybni *Laetiporus sulphureus* i *Trametes versicolor* na pożywkach o wzrastającym stężeniu rezorcynolu (kolor szary wskazuje istnienie istotnej pod względem statystycznym różnicy – test NIR przy poziomie ufności 95%)

Averaging the results for the two test fungi, the most efficient substances included: Eugenol (4.0), Isoeugenol (4.5), Pyrogallol (5.0), 2-furaldehyde (5.0) and Resorcinol (5.0). In case of Pyrogallol it is not ruled out that its real fungitoxicity is higher towards both *L. sulphureus* (the speed of mycelial growth on media of concentration 100 and 50 ppm were not statistically different), and especially towards *T. versicolor* for which no statistical differences between the speed of growth of mycelium colony on medium of concentration of active substance equal to 100 ppm and the seven smaller concentrations (down to 0.1 ppm inclusive) were recorded – it can mean that the fungitoxicity class of that substance towards *T. versicolor* is lower (i.e. it is more toxic) and can be 4; 3 or even 2 (Fig. 4). Similar relations are visible also in case of 2-Furaldehyde, especially towards *L. sulphureus* (real fungitoxicity class of this preparation towards *L. sulphureus* can be 4 and even 3; Fig. 5) and Resorcinol, especially for *T. versicolor* mycelium for which the real class of fungitoxicity can be equal to 4 (Fig. 6).

DISCUSSION

The results obtained in the test described above have proven that most of tested phenolic compounds naturally occurring in wood are characterized by minor degree of influence on mycelial growth, that using the terminology shown in Table 1 can be described as 'little toxic' to 'very little toxic'. Only two substances – Eugenol and Isoeugenol – demonstrated slightly higher efficiency as fungicides, that allows to describe them as 'moderately toxic. The results indicate that the tested phenolic substances naturally occurring in wood are characterized by a limited ability to stop the mycelial growth in *in vitro* conditions and are no match in this regard for substances practically used for wood protection nowadays, especially modern, compound systemic fungicides. As an example synthetic fungicidal substances tested in the same manner such as Falcon 460 EC containing Spiroxamine (250 g/l), Terbuconazol (167 g/l) and Triadimenol (43 g/l) as well as Preventol R 80 that contains Benzalkonium (78-82%) indicated respectively 1 and 2 class of fungitoxicity when tested on both *Trametes versicolor* and *Laetiporus sulphureus* mycelium [Zarzyński 2004].

Fungistatic capabilities of a given chemical substance understood according to the traditional interpretation of the term consists of its ability to permeate directly into mycelium cells and block the processes of breathing, disabling the possibility of exchanging substances with the surroundings and preventing the synthesis of proteins, nucleic acids and ergosterol [Borecki 1996, Kryczyński 2000]. The characteristics of this kind are usually easily detectable in medium tests, such as the ones conducted in experiment described in the present study. Therefore, the results obtained theoretically indicate a lack of potential usefulness of tested phenolic substances and their mixtures for practical use in trees and wood protection against saprotrophic and parasitic fungi that are the cause of depreciation of that resource. However, as wood is a material of a very complex structure and chemical composition, it is not ruled out that some of these substances introduced directly into the material can demonstrate much higher efficiency in protecting the wood against fungal decay. According to literature [Rayner and Boddy 1988, Evensen et al. 2000] phenolic substances can connect with proteins present in the wood, lowering at the same time the nutritional value of wood tissue attacked by the pathogen. Therefore it is not impossible that some of the tested chemical compounds

introduced directly into the wood, especially the wood of live trees, can be characterized by exactly that mechanism of operation, 'impregnating' the wood in natural way and efficiently protecting it from being putrefied by the fungi despite the lack of having typical fungistatic abilities that are possible to be detected in *in vitro* conditions.

CONCLUSIONS

Almost all of the tested phenolic compounds naturally occurring in wood most probably do not show significant abilities to stop the growth of mycelium on media (however in some of the cases the results of the statistical analysis indicate, that in reality the abilities can be more developed than it appears from the experiment results).

Only Eugenol and Isoeugenol have scarce fungistatic characteristics, however they do not seem to be sufficient from the point of view of their potential usage for practical fighting off wood decaying fungi.

All of the tested natural phenolic compounds occurring in wood and their mixtures are much less efficient in terms of fungitoxicity than synthetic substances practically used for protection of plants and wood nowadays, especially compound systemic fungicides of the new generation.

It cannot be ruled out that despite the anticipated lack of potential fungistatic *in vitro* abilities in the traditional meaning of the term (that is the ability to permeate directly into mycelium cells and block the processes of breathing, disabling the possibility of exchanging substances with the surroundings and preventing the synthesis of proteins, nucleic acids and ergosterol) some of the tested chemical compounds can be characterized by a much higher efficiency in this regard in wood, especially in wood of live trees (*in vivo*). However the verification of that theory requires performing further laboratory and field tests.

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OCENA STOPNIA FUNGITOKSYCZNOŚCI *IN VITRO* WYBRANYCH SUBSTANCJI O CHARAKTERZE FENOLOWYM NATURALNIE WYSTĘPUJĄCYCH W DREWNIE Z ZASTOSOWANIEM METODY POŻYWKOWEJ AG

Streszczenie. W pracy przedstawiono przebieg i wyniki badań stopnia fungitoksyczności wybranych substancji fenolowych występujących w drewnie, które mogą być odpowiedzialne za naturalną odporność tego surowca na rozkład przez grzyby. Badaniom poddano łącznie 14 substancji: eugenol, kwas wanilinowy, izoeugenol, cykloheksanon, rezorcynol, syringaldehyd, 2,6-dimetoksyfenol, pirogalol, kwas 4-metoksybenzoesowy, 2-furaldehyd, furanon, 4-allyl-2,6-dimetoksyfenol, tetrametylo-4-butanodiaminę i 3',5'-dimetoksyacetofenon oraz sześć różnych mieszanin tych związków naśladujących ich naturalne współwystępowanie w drewnie. W badaniach posłużono się zmodyfikowaną metodą pożywkową AG opartą na zastosowaniu serii płytek z pożywką o różnym nasyceniu testowanym środkiem. Jako grzyby testujące posłużyły *Trametes versicolor* i *Laetiporus sulphureus*. Łącznie wykonano 20 wariantów doświadczenia na 2400 płytkach. Na tej podstawie określono stopień fungitoksyczności poszczególnych substancji i oceniono ich potencjalną przydatność do zastosowania w praktycznej ochronie drzew i drewna.

Słowa kluczowe: testy pożywkowe, *Laetiporus sulphureus*, *Trametes versicolor*, naturalna ochrona drewna, związki fenolowe

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