

PRACE NAUKOWE – RESEARCH PAPERS

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ACTIVITY OF NEW AMMONIUM IONIC LIQUIDS AGAINST FUNGI CAUSING WOOD MOULDING

*The results of the tests of the effectiveness of the action of new, not yet described in the literature group of biocidal ionic liquids were synthesised and presented. Those ionic liquids are derivatives of the leading structure, i.e. didecyldimethylammonium nitrate, and demonstrate strong action against mould fungi. Natural quaternary ammonium salts, mainly extracts from coconut and soybean, and from vegetable fats, were the basis for syntheses. Mycological tests were carried out on the wood of pine *Pinus sylvestris* L. (sapwood) in accordance with the method assumed binding for the assessment of biocide efficacy.*

Keywords: ionic liquids, biocidal activity, wood, moulds

Introduction and aim of the research

Wood is very susceptible to depreciation and processes of biological degradation caused by microorganisms. Microorganisms present in air are undesirable

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biological factor which, if allowable concentrations assumed for a certain environment are exceeded, poses a threat to people. Mould fungi account for around 70% of the whole air micro flora. In residential buildings a few dozen species of bacteria, over 400 species of moulds and a dozen or so species of fungi causing decay of wood and wood-based materials [Barabasz, Pikulicka-Dziurman 2009] may be found. Microorganisms settling so-called “sick buildings” are isolated from air, walls, and from furniture as well. Wood, which still is one of the most common furniture maker’s and structural materials in construction industry, unfortunately is an excellent substratum for the growth of fungi, which is the reason why it becomes the main source of mould in indoor air.

The main factor stimulating the growth of mould fungi in apartments is an elevated humidity of environment, i.e. above 60% of air relative humidity, although we know some species for which humidity of 30–40% is sufficient [Gutarowska, Jakubowska 2001].

Tests of microbiological contamination of usable rooms proved a high and diverse level of microbiological contamination in the tested air. The following moulds were identified: *Rhizopus*, *Penicillium*, *Aspergillus*, *Cladosporium* and *Alternaria* [Stach et al. 2004].

Moulding of wood is a process brought about by many species of fungi, most often growing on the surface. Usually moulding does not cause any stronger deterioration of physical and chemical properties; however it is responsible for depreciation of wood through change of its colour and making its surface pattern blurry, thus for depreciation of wood commercial value and its competitiveness in relation to other technical materials. Infected wood cannot be used in line with its intended use not only due to worsening of its aesthetics, but also because of allergenic properties of many of moulds found on it, which surely have a negative influence on health of people staying in rooms. Mould fungi and their secondary metabolites prove to be toxic to the organisms of people and animals. Numerous tests proved that they are directly responsible for many conditions of respiratory system and skin, part of cancerous diseases or numerous allergies. People who are exposed to mould infected materials for a longer period may have such symptoms of infections and allergies as: rhinitis, sinusitis, laryngitis, bronchitis, alveolitis, conjunctivitis, dermatological lesions, reduction of immune system resistance, chronic fatigue syndrome, and in unusually extreme cases damage to immune system may lead to death [Grajewski, Twarużek 2004]. Bearing in mind that wood being one of more popular materials found in human nearest environment, is at the same time a good substratum for mould growth, it requires protection that would be simultaneously solid and harmless to human. With a view to achieve that, fungicides limiting possibilities of fungi attack on wood are introduced into wood [Pernak et al. 2004, 2006; Cybulski et al. 2008; Zabielska-Matejuk 2004; Zabielska-Matejuk et al. 2009].

Diversity of species and the resistance of mould fungi to chemical preparations poses a problem when structures of effective active substances combating this group of imperfect fungi are being designed. This paper presents attempts to develop new, not yet described in the literature biocidal ionic liquids that are derivatives of the lead structure, i.e. didecyldimethylammonium nitrate, and demonstrate strong action against mould fungi. Natural quaternary ammonium salts, mainly extracts from coconut and soybean, and from vegetable fats, were the basis for the syntheses.

The aim of the research was to develop a manner of synthesising new ionic liquids and determine their efficacy against species of mould fungi that overgrow wood and wood-based materials.

Test materials and methods

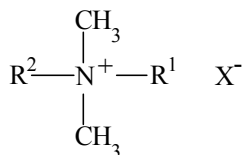
Syntheses

New, not yet described in the literature biocidal ionic liquids with ammonium cation and organic herbicide anions were synthesised: [Ciech][1], [Ciech][3], [Rok][1], [Rok][2], [BA][Glif], as well as ionic liquids with natural cation and nitrate anion: [Eth C/12][NO₃], [Arq1230][NO₃] and [Arq C35][NO₃] (table 1.)

The synthesis of the above-mentioned ionic liquids consisted in replacement of halide anion with herbicide anion or nitrate(V) anion. The reactions were carried out in water environment with a little surplus of inorganic salt.

By selecting adequate surpluses of inorganic salts participating in the synthesis and regulating the temperature and time of the ion exchange process, very pure ionic liquids, whose effectiveness not infrequently reached 98–99%, were obtained.

In order to confirm the structure of the new ionic liquids, analyses using thin layer chromatography (TLC) and proton and carbon spectra of nuclear magnetic resonance (¹H NMR and ¹³C NMR) were carried out, as well as elementary analyses. The content of ionic liquid in the product was determined using two phase titration method in chloroform-water system. The structures of ionic liquids are presented in Diagram 1 and the description is given in table 1.



R¹, R² – alkyl C₈-C₁₈, X – NO₃, herbicide,

Table 1. Tested ionic liquids
Tabela 1 . Badane ciecze jonowe

Ionic liquid <i>Ciecz jonowa</i>	Content of cation active substance [%] <i>Zawartość substancji aktywnej [%]</i>	Solvent <i>Rozpuszczalnik</i>	Description <i>Opis</i>
[Ciech][1]	88.0	isopropanol <i>izopropanol</i>	modified structure of CIECH group products <i>zmodyfikowana struktura produktów grupy CIECH</i>
[Ciech][3]	98.0	isopropanol <i>izopropanol</i>	modified structure of CIECH group products <i>zmodyfikowana struktura produktów grupy CIECH</i>
[Rok][1]	95.0	isopropanol/water 1:1 <i>izopropanol/woda 1:1</i>	synthesis from a market product of PCC Rokita S.A. Company <i>synteza z produktu rynkowego firmy PCC Rokita S.A.</i>
[Rok][2]	99.0	isopropanol/water 1:1 <i>izopropanol/woda 1:1</i>	synthesis from a market product of PCC Rokita S.A. Company <i>synteza z produktu rynkowego firmy PCC Rokita S.A.</i>
[BA][Glif]	95.0	water <i>woda</i>	ionic liquid with anion containing herbicide <i>ciecz jonowa z anionem zawierającym herbicyd</i>
[Arq1230][NO ₃]	75.0	water <i>woda</i>	nitrate(V) with dodecyltrimethylammonium cation <i>azotan(V) z kationem dodecylotrimetyloamoniowym</i>
[Arq C35] NO ₃	98.0	water <i>woda</i>	nitrate(V) with cation obtained from coconut oil <i>azotan(V) z kationem uzyskanym z oleju kokosowego</i>
[EthC/12][NO ₃]	99.0	water <i>woda</i>	nitrate(V) with cation obtained from natural vegetable products (coconut oil ethoxylates) <i>azotan(V) z kationem uzyskanym z naturalnych produktów roślinnych (etoksylatów oleju kokosowego)</i>

Method for testing wood resistance to mould fungi

Mycological tests were carried out in accordance with the method assumed binding for the assessment of biocide efficacy against mould fungi and described in ITB Instruction 355/98 [Ochrona drewna ... 1998]. Water solutions, alcohol solutions (isopropanol) or water-alcohol solutions (isopropanol-water 1:1) of the tested ionic liquids were applied on the samples of pine wood (*Pinus sylvestris* L. (sapwood)) of the dimensions of 40 × 40 × 4 mm. The applied amount was 15, 25, 50, 100 and 150 g of pure active substance per 1 m² of the wood surface. After conditioning the protected samples as well as the control samples were exposed to the action of two sets of fungi. The samples were placed on agar culture medium in Petri dishes and then infected by spraying them with spore water made from the following species of mould fungi:

Set I – *Aspergillus niger* V. Tieghem, *Penicillium funiculosum* Thom, *Paecilomyces varioti* Rainier, *Trichoderma viride* Persoon ex Fries, *Alternaria tenuis* Link ex Fries,

Set II – *Chaetomium globosum* Kunze.

The test material was put for 4 weeks into an incubator where the temperature was 27±2°C and relative humidity 90%. Simultaneously the vitality of spores on the control dishes with agar culture medium was checked. Unprotected pine wood was used as reference material. The assessment of mycelium growth was carried out according to a 4-degree assessment scale:

0 – no visible under the microscope growth of fungi on a sample,

1 – trace growth of fungi on a sample, poorly visible to the naked eye, but well visible under the microscope, or visible to the naked eye growth limited only to the brims of a sample,

2 – visible to the naked eye growth of fungi on a sample, but less than 15% of the surface covered by fungus,

3 – over 15% of the surface covered by fungus visible to the naked eye.

The final result of a sample coverage by mould was the arithmetic mean from the assessments of six test samples from each set.

The control samples should demonstrate fungi growth on over 75% of the surface of each sample.

Test results and discussion

The results of determinations of the degree of wood coverage by the mould fungi are presented in tables 2 and 3 and in fig. 1.

The degree of mould coverage of the wood protected with [Ciech][1], [BA][Glif] ionic liquids after 4-week action of the mould fungi mixture (set I)

and where the application was 25 g/m² was 0, which proves very high activity of the compounds with herbicide anion against the mixture of test fungi.

Table 2. The resistance of pine wood (sapwood) protected with the ionic liquids to the action of the mould fungi mixture – Set I

Tabela 2. Odporność drewna sosny (biel) zabezpieczonego cieczami jonowymi na działanie mieszaniny grzybów pleśniowych – Zestaw I

Ionic liquid <i>Ciecz jonowa</i>	Ionic liquid application on the wood surface <i>Naniesienie cieczy jonowej na powierzchnię drewna</i>				
	15 [g/m ²]	25 [g/m ²]	50 [g/m ²]	100 [g/m ²]	150 [g/m ²]
	Surface coverage degree <i>Stopień porośnięcia powierzchni</i>				
[Ciech][1]	0.3	0.0	0.0	0.0	0.0
[Ciech][3]	1.0	1.0	0.8	0.2	0.0
[Rok][1]	0.8	0.3	0.3	0.2	0.0
[Rok][2]	2.0	0.7	0.0	–	–
[BA][Glif]	1.8	0.0	0.0	–	–
[Arq1230][NO ₃]	1.8	0.7	0.3	–	–
[Arq C35][NO ₃]	2.3	0.7	0.0	–	–
[Eth C/12][NO ₃]	3.0	2.5	0.5	0.5	0.3

Table 3. The resistance of pine wood (sapwood) protected with the ionic liquids to the action of *Chaetomium globosum* fungus – Set II

*Tabela 3. Odporność drewna sosny (biel) zabezpieczonego cieczami jonowymi na działanie grzyba *Chaetomium globosum* – Zestaw II*

Ionic liquid <i>Ciecz jonowa</i>	Ionic liquid application on the wood surface <i>Naniesienie cieczy jonowej na powierzchnię drewna</i>				
	15 [g/m ²]	25 [g/m ²]	50 [g/m ²]	100 [g/m ²]	150 [g/m ²]
	Surface coverage degree <i>Stopień porośnięcia powierzchni</i>				
[Ciech][1]	0	0	0	0	0
[Ciech][3]	0.8	0.5	0	0	0
[Rok][1]	0.3	0.2	0	0	0
[Rok][2]	0	0	0	–	–
[BA][Glif]	0	0	0	–	–
[Arq1230][NO ₃]	0	0	0	–	–
[Arq C35][NO ₃]	0	0	0	–	–
[Eth C/12][NO ₃]	1.8	0.7	0	0	0

In the case of the rest of the ionic liquids with ammonium cation and organic herbicide anions ([Ciech][3], [Rok][1], [Rok][2]) and the samples protected with the ionic liquids with natural cation and nitrate anion ([Arq1230][NO₃] [ArqC35][NO₃]) already the application of 25 g/m² resulted in the situation where the maximum coverage degree did not exceed 1. Such result allow classification of the protected wood as a material that is practically resistant to mould fungi (class 2).

The fact that wood was well protected with [Ciech][1], [Ciech][3] and [Rok][1] liquids already at the application level of 15 g/m² should be well noticed. The mean mould coverage degree ranged from 0.3 to 1.0.

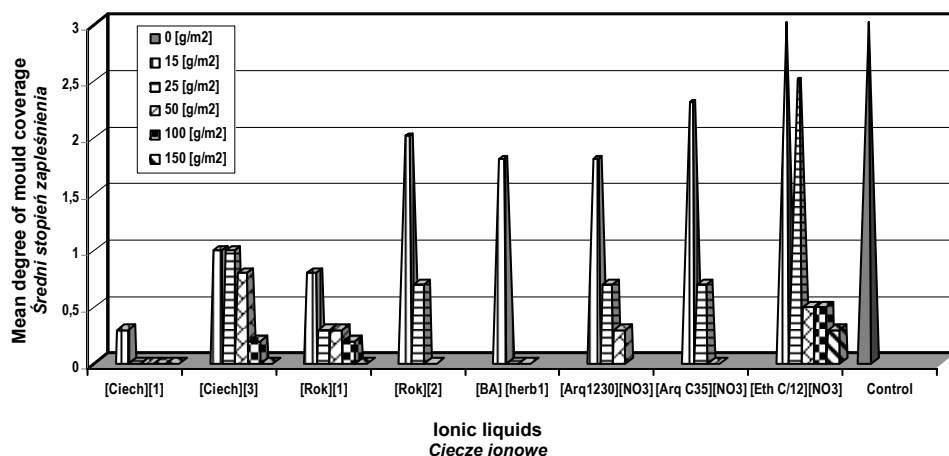


Fig. 1. The results of determination of the resistance of pine wood protected with the ionic liquids to the action of the mould fungi – Set I – Mixture

Rys. 1. Wyniki oznaczania odporności drewna sosny zabezpieczonego cieczami jonowymi na działanie grzybów pleśniowych – Zestaw I – Mieszanina

In the case of *Chaetomium globosum* fungus the degree of coverage was 0 for application level of 50 g/m², which indicates exceptional efficacy of the tested ionic liquids against that species.

The pine control wood demonstrated the 3. degree of coverage by mould as a result of the action of both sets of the test fungi.

Conclusions

1. The seven tested ionic liquids demonstrated strong anti-mould action. In the case of *Chaetomium globosum* fungus (set II) already the lowest application (15 g/m²) was sufficient to protect the wood effectively.

2. The ionic liquids with ammonium cation and organic herbicide anions ([Ciech][1], [BA][Glif]) demonstrated the strongest action against the mould fungi. Those ionic liquids completely inhibited the growth of the mould mixture (set I) on the surface of protected material already when the application was 25 g per m² of wood.

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AKTYWOSĆ NOWYCH AMONIOWYCH CIECZY JONOWYCH WOBEC GRZYBÓW WYWOŁUJĄCYCH PLEŚNIENIE DREWNA

Streszczenie

Drewno użytkowane w warunkach wysokiej wilgotności ulega deprecjacji i procesom destrukcji biologicznej wywołanej działaniem mikroorganizmów. Szczególnie jest podatne na atak grzybów pleśniowych, wywołujących powierzchniowe przebarwienia, co pogarsza estetykę drewna oraz obniża jego konkurencyjność w stosunku do innych materiałów technicznych. Różnorodność gatunkowa oraz oporność grzybów pleśniowych na preparaty chemiczne jest problemem przy opracowaniu struktur skutecznych substancji czynnych, zwalczających tę grupę grzybów niedoskonałych.

Zsyntezowano nową, nieopisaną w literaturze, grupę biobójczych cieczy jonowych, pochodnych struktury wiodącej – azotanu didecyłodimetyloamoniowego, wykazujących silne działanie w stosunku do grzybów-pleśni. Bazą dla syntez były czwartorzędowe sole amoniowe pochodzenia naturalnego, głównie ekstrakty z kokosa, soi oraz tłuszczów roślinnych. Badania mikologiczne wykonano na drewnie sosny *Pinus sylvestris* L.(biel), zgodnie z metodą przyjętą jako obowiązującą w ocenie skuteczności działania biocydów. Opracowane ciecze jonowe wykazały aktywność wobec gatunku *Chaetomium globosum*, oraz mieszaniny: *Aspergillus niger*, *Penicillium funiculosum*, *Alternaria alternata*, *Paecilomyces varioti*, *Trichoderma viride*. Jako najsilniej działające ciecze jonowe wymienić należy związki o kationach: didecyłodimetyloamoniowym, benzalkoniowym, dodecylo-trimetyloamoniowym i cocotrimetyloamoniowym oraz szeregu anionów organicznych i nieorganicznych (również o właściwościach herbicydowych).

Słowa kluczowe: ciecze jonowe, aktywność grzybobójcza, drewno, pleśnienie