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ИССЛЕДОВАНИЕ ДЕЙСТВИЯ ВОДОРАСТВОРИМЫХ ГУАНИДИНСОДЕРЖАЩИХ (СО)ПОЛИМЕРОВ НА ASPERGILLUS NIGER И ОЦЕНКА ИХ ЭФФЕКТИВНОСТИ

С.А. Стельмах, Н.М. Гаркушева, Е.В. Лаврентьева, М.Н. Григорьева, О.С. Очиров, В.О. Окладникова

Сергей Александрович Стельмах (ORCID 0000-0003-3392-5600)*, Наталья Михайловна Гаркушева (ORCID 0000-0002-6404-3021), Мария Николаевна Григорьева (ORCID 0000-0003-4184-2805), Олег Сергеевич Очиров (ORCID 0000-0002-2317-4105), Валерия Олеговна Окладникова (ORCID 0000-0002-0903-8780)

Байкальский институт природопользования СО РАН, ул. Сахьяновой, 6, Улан-Удэ, Российская Федерация, 670047
E-mail: s_stelmakh@bk.ru*, nmgarkusheva@gmail.com, gmn_07@bk.ru, ochirov.o.s@yandex.ru, lera-okladnikova@mail.ru

Елена Владимировна Лаврентьева (ORCID 0000-0002-2500-103X)

Институт общей и экспериментальной биологии СО РАН, ул. Сахьяновой,6, Улан-Удэ, Российская Федерация, 670047
E-mail: lena_l@mail.ru

В представленной работе рассмотрено действие модифицированных водорастворимых гуанидинсодержащих (со)полимеров на штамм плесневого гриба Aspergillus niger (A. niger). Установлено, что основной компонент широкоиспользуемых биоцидов полигексаметиленгуанидин гидрохлорид (ПГМГх) наименее эффективно подавляет рост плесневого гриба, а увеличение содержания октаметиленовых фрагментов в макромолекулярной структуре, то есть увеличение гидрофобности, приводит к возрастанию фунгицидного действия и полностью подавляет рост клеток гриба. Проведены лабораторные эксперименты по изучению действия (со) полимеров при добавлении их в питательную среду в чашках Петри, а также при обработке поверхности их растворами разной концентрации реального объекта – здоровой древесины Сосны обыкновенной (Pinus sylvestris). Определено, что максимальное подавление роста A. niger достигается на питательной среде, содержащей 30 и 50% октаметилендиамина, что сопоставимо с данными, полученными при оценке фунгицидного действия на древесине Pinus sylvestris. В ходе исследования, полученные зависимости позволяют предположить механизм взаимодействия клеточной стенки гриба с (со)полимером, где на первом этапе воздействия происходит сорбция макромолекул полимера оболочкой стенки клетки гриба за счет содержащихся в ней белков – «адгезинов» с последующим нарушением целостности клетки и завершается осмотическом лизисом. Дана оценка эффективности исследуемых соединений и произведена их классификация согласно степени защитных свойств к поражению плесневелыми грибами древесины. Согласно классификации, исследуемые модифицированные водорастворимые гуанидинсодержащие (со)полимеры являются эффективными и высокоэффективными средствами по защищающей способности противостоять заражению поверхности древесины плесневыми грибами.

Ключевые слова: водорастворимые гуанидинсодержащие (со)полимеры, гидрофобность, конформация, антисептик, Aspergillus niger, фунгицидная активность, дереворазрушающие грибы

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THE IMPACT OF WATER-SOLUBLE GUANIDINE-CONTAINING (CO)POLYMERS ON ASPERGILLUS NIGER AND THE ASSESSMENT OF THEIR EFFICIENCY

S.A. Stelmakh, N.M. Garkusheva, E.V. Lavrentieva, M.N. Grigor'eva, O.S. Ochirov, V.O. Okladnikova

Sergey A. Stelmakh (ORCID 0000-0003-3392-5600)*, Natalya M. Garkusheva (ORCID 0000-0002-6404-3021), Maria N. Grigor'eva (ORCID 0000-0003-4184-2805), Oleg S. Ochirov (ORCID 0000-0002-2317-4105), Valeria O. Okladnikova (ORCID 0000-0002-0903-8780)

Baikal Institute of Nature Management of SB of the RAS, Sakhyanova st., 6, Ulan-Ude, 670047, Russia
E-mail: s_stelmakh@bk.ru*, nmgarkusheva@gmail.com, gmn_07@bk.ru, ochirov.o.s@yandex.ru, lera-okladnikova@mail.ru

Elena V. Lavrentieva (ORCID 0000-0002-2500-103X)

Institute of General and Experimental Biology of the Siberian Branch of the RAS, Sakhyanova st., 6, Ulan-Ude, 670047, Russia
E-mail: lena_l@mail.ru

The presented work examines the effect of modified water-soluble guanidine-containing (co)polymers on a strain of the mold Aspergillus niger (A. niger). It has been established that the main component of widely used biocides, polyhexamethylene guanidine hydrochloride (PHMGgx), is the least effective at inhibiting the growth of mold fungi, and an increase in the content of octamethylene fragments in the macromolecular structure, that is, an increase in hydrophobicity, leads to an increase in the fungicidal effect and completely suppresses the growth of fungal cells. Laboratory experiments were carried out to study the effect of (co)polymers when they were added to a nutrient medium in Petri dishes, as well as when the surface was treated with solutions of different concentrations of a real object - healthy Scots Pine (Pinus sylvestris) wood. It was determined that the maximum inhibition of A. niger growth is achieved on a nutrient medium containing 30 and 50% octamethylenediamine, which is comparable to the data obtained when assessing the fungicidal effect on Pinus sylvestris wood. In the course of the study, the obtained dependences suggest a mechanism for the interaction of the fungal cell wall with the (co)polymer, where at the first stage of exposure, sorption of polymer macromolecules by the fungal cell wall membrane occurs due to the proteins contained in it – «adhesins», followed by a violation of the integrity of the cell and ends with osmotic lysis. The effectiveness of the studied compounds was assessed and their classification was made according to the degree of protective properties against wood damage by moldy fungi. According to the classification, the studied modified water-soluble guanidine-containing (co)polymers are effective and highly effective means of protective ability to resist infection of the wood surface by mold fungi.

Key words: water-soluble guanidine-containing (co)polymers, hydrophobic properties, conformation, antiseptic, Aspergillus niger, fungicidal activity, wood-decay fungus

INTRODUCTION

Wood has been used as one of the key construction materials for thousands of years. Despite the significant progress in construction material chemistry and the emergence of new composite materials, wood still plays a leading role in the construction of low-rise buildings. Following the Rosstat data for 2010-2021, housing development has been growing steadily both in cities and rural areas. Of 87 million square meters of housing constructed, 22% used wood as the wall material [1].

Wood processing and wood product manufacturing have also been on a steady rise. For instance, the volume of timber processing reached 32.3 million m^3, which is 13% higher than the 2018 figure [2]. Although wooden furniture is the most popular kind of product, wood is also used for the production of floating items and even toys, which is especially relevant when the production of eco-friendly items peaks.

However, this material is subjected to various ambient factors during operation that may gradually destroy it. These include humidity, acidity, temperature, decomposer germs, etc. The problem of biodeterioration of wood as a production material rather than living trees can be attributed to the fact that cellulose makes a great organic substrate and food for different microorganisms, especially fungi. Microorganisms cause stains of different colors and mold to appear on the wooden surfaces which results in the reduction of its durability [3, 4]. Besides, if mold appears in a house, it starts to grow and cover different surfaces. Humans that live in the contaminated building experience the harmful impacts of mold. Mold fungi are dangerous because of their mycotoxins that suppress or poison living organisms. They may cause dizziness, headaches, skin and inhalant allergies, that are hard to diagnose and treat, as well as other health disorders, such as migraines, runny nose, otitis, bronchitis, rhinitis, asthma, cardiac disorders, and mycotoxicosis. People with reduced immune system activity may get mold to damage their internal organs [5]. Mold formed by *Aspergillus* fungi is especially dangerous because its spores contain aflatoxin that, apart from the toxic action, may cause hepatic cancer [6].

Currently, various wood-protection antiseptic is available, but they were developed several decades ago, which results in the gradual adaptation of molds to them [7, 8]. This calls for the development and testing of new and more efficient solutions or alternative wood product preservation methods. The substance hazard category is an important factor in the use of protective solutions. It has to be at least Category 4 [5].

Properties like high biocidal activity, low toxicity (Hazard Category 4), inexpensive monomers, simple synthesis, low corrosive activity, ability to maintain antiseptic properties over a long time, as well as extensive macromolecular design opportunities make new modified water-soluble polyguanidines a promising alternative to the existing solutions [9].

EXPERIMENTAL METHODS

Previous research [10, 11] determined the biocidal activity of modified polyguanidines compared to opportunistic pathogenic microorganisms. The prominent antimicrobial action against the *Candida* fungi among others demonstrated the potential fungicidal activity of guanidine-containing (co)polymers. To determine that, we assessed the biocidal action of PHMGhc and PHMGhc-based copolymers against the *A. niger* mold fungus. All of the polymer products were synthesized in the hot melt under different molar ratios of monomers in the same way as before [12].

The antifungal properties were assessed using the serial dilution technique in agar [13]. Note that this article determines the biocidal activity of (co)polymers used in wood treatment against mold fungi following GOST 30028.4.-2006. Protective solutions for wood [14].

The synthesis of (co)polymers

All of the (co)polymers were obtained using the polycondensation method for the hot melt of the following bifunctional amines: hexamethylenediamine (HMDA), octaethylenediamine (OMDA), and guanidine hydrochloride (GHC) in two stages lasting 6 hours and at different ratios presented in Table 1. For the experiments, we prepared low-concentration water solutions similar to the existing concentrations of popular fungicidal solutions.

Table 1

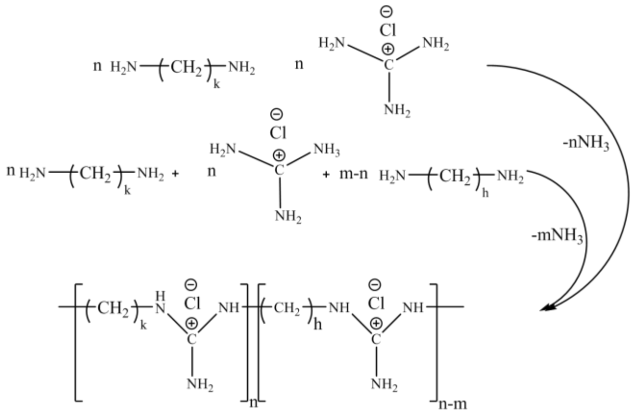
The characteristics of (co)polymers and the concentrations of their water solutions

Таблица 1. **Характеристики (со)полимеров и концентрации их водных растворов**

No.	Concentration, %	The molar ratio of monomers			[η], dl/g
		HMDA	OMDA	GHC	
1	0.5	0.9	0.1	1.0	0.043
2	1.0				
3	2.0				
4	0.5	0.7	0.3	1.0	0.041
5	1.0				
6	2.0				
7	0.5	0.5	0.5	1.0	0.038
8	1.0				
9	2.0				
10	0.5	1.0	-	1.0	0.045
11	1.0				
12	2.0				

The first stage of synthesis took place at 165 °C for 2.5 h, after which the temperature was increased to 185 °C [12].

Reaction schemes:



The structures of all of the obtained statistical polyguanidines were studied using physical and chemical methods and described in [10, 11].

Preparing the test organism

The freeze-dried cells of the test organism (a strain of the *A. niger* mold fungus from All-Russian collection of industrial microorganisms (Genetika, Moscow, Russia) were inoculated on Saburo broth (State Research Center for Applied Biotechnology and Microbiology, Obolensk) using the methodology of Genetika, Moscow. After that, the culture of *A. niger* grown in the Sabouraud dextrose broth for 2 days at 24 °C reached the concentration of 2×10^9 CFU/ml using the optic turbidity standard [15] with a Cecil – 1021 spectrophotometer (UK).

RESEARCH METHODS AND TECHNIQUES

The biocidal properties were assessed using the serial dilution technique in agar [13]. The obtained cell-rich fluid of *A. niger* contained ~10^5 CFU/ml. Under aseptic conditions, we mixed thoroughly 20 mg of Sabouraud-maltose-agar heated to 50 °C and 0.05; 0.025; 0.0125 ml of the 0.1% solution of the polymers in question directly in the double dish. Simultaneously, we prepared dishes without fungicidal solutions to reference the growth of the test organism. When the agarized broth consolidated and the dishes dried, we inoculated 0.1 ml of *A. niger* cell fluid on the prepared solid nutrient media five times and incubated them in a thermostat at 24 °C for 48 h to calculate the surviving CFU (N_{av}).

To determine the fungicidal action of (co)polymers following GOST 30028.4.-2006 [14], we prepared the working fluid of *A. niger* containing 1 million spores in the calculated volume. The measured amounts of fungus fluids were poured into a beaker and complemented with sterile distilled water until the volume reached (100 ± 1) cm^3.

The experiment was carried out in desiccators filled with sawdust to ¼ of their height and moisturized to (70 ± 5)% in advance. When the desiccators were filled, the sawdust was contaminated with the working fungus fluid using a sprayer. The desiccators were placed in a room with a temperature of 25 ± 2 °C and kept there for 14 days before the experiment to let *A. niger* grow and develop.

The fungicidal activity of polymers was assessed on healthy wood samples (the albur of *Pinus sylvestris*) sized 10×55×75 mm. On the day of the experiment, we prepared the wood samples for the tests. To do this, we put numbered wood samples into the prepared (co)polymer solutions and kept them there for 1 min. We used PHMGhc as the reference. The experiment was attempted three times.

The wood samples treated with water solutions of (co)polymers, as well as the reference (untreated) sample, were placed in desiccators filled with contaminated sawdust. The test lasted for 15 days. The state of the samples was assessed visually, and damaged areas were measured with a ruler on the 5th, 10th, and 15th day, according to the methodology.

RESULTS AND DISCUSSION

The serial dilution experiment in agar (Fig. 1) used (co)polymers with a 1% concentration, as prescribed by the methodology, namely samples 2, 5, 8, and 11 (Table 1) and the reference sample.

The data obtained are shown in Table 2. Fig. 1 shows the photographs of one of the experimental sequences with content of (co)polymers of 0.0125 ml.

The data in Table 1 show that PHMGhc (No. 11) has the smallest efficiency in suppressing the growth of the mold fungus, while the ratio between the reference and the CFU number is practically unaffected by its content in the nutrient medium. As the content of octamethylene fragments in the macromolecular structure increases, i.e. as the hydrophobic properties of copolymers increase, their biocidal activity increases dramatically, which is especially obvious when their content is high (0.05 ml). Sample 8 completely suppressed the growth of *A. niger* in all of the cases.

After that, we conducted a live experiment following GOST 30028.4.-2006 Protective Solutions for Wood.

Table 2

The reduction of *A. niger* depending on the (co)polymer sample and its content in the nutrient medium

***Таблица 2.* Редукция *A. niger* в зависимости от образца (со)полимера и его содержания в питательной среде**

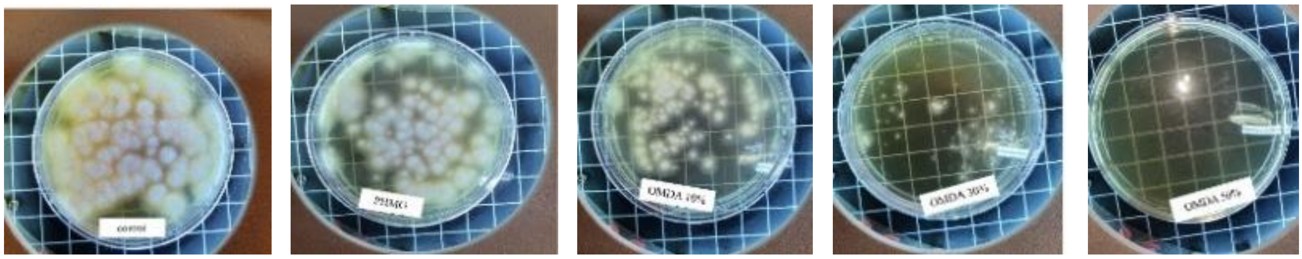
Sample No.	0.05 ml		0.025 ml		0.0125 ml	
	$N_{av}\cdot10^3$	Ratio**	$N_{av}\cdot10^3$	Ratio**	$N_{av}\cdot10^3$	Ratio**
Control*	95	-	106	-	71	-
11	86	1.10	88	1.20	61	1.16
2	5	19.00	52	2.00	59	1.20
5	0	-	0	-	55	1.29
8	0	-	0	-	0	-

Notes: * nutrient medium without (co)polymers

** the ratio of the CFU number in the reference sample to the CFU number in the experimental sample

Примечания: * питательная среда без добавления (со)полимеров

** отношение количества КОЕ контроля к количеству КОЕ в образце



Control PHMGhc 10% OMDA 30% OMDA 50% OMDA

Fig. 1. The photographs of the growth of *A. niger* colonies in the nutrient medium with a (co)polymer content of 0.0125 ml
Рис. 1. Фотографии роста колоний *A. niger* на питательной среде с содержанием (со)полимеров 0.0125 мл

Fig. 2 shows the dynamics of the biocidal activity of the compounds in question (Samples 1-12, Table 1, 13 is the untreated sample).

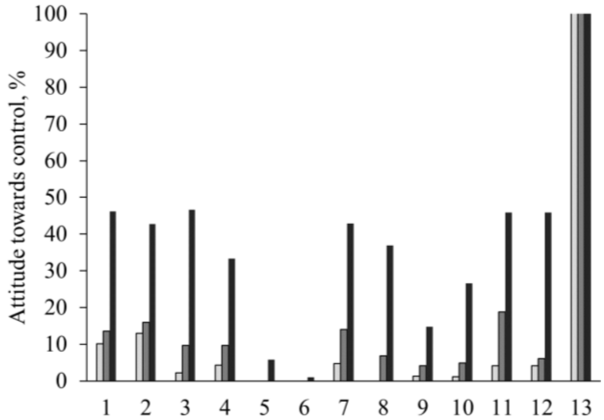


Fig. 2. The dynamics of the fungicidal properties of PHMGhc and its copolymers compared to the untreated sample (control) in %: - 5 days of exposure; - 10 days of exposure; - 15 days of exposure. 1 – 0.5% OMDA – 10%; 2 – 1,0% OMDA – 10%; 3 – 2.0% OMDA – 10%; 4 – 0.5% OMDA – 25%; 5 – 1.0% OMDA– 25%; 6 – 2.0% OMDA – 25%; 7 – 0.5% OMDA – 50%; 8 – 1.0% OMDA – 50%; 9 – 2.0% OMDA – 50%; 10 – PHMGhc – 0.5%; 11 – PHMGhc – 1%; 12 – PHMGhc – 2.0%; 13 – Control
Рис. 2. Динамика фунгицидной способности ПГМГгх и его сополимеров по отношению к необработанному образцу (контроль) в %: - 5 сут. воздействия; - 10 сут. воздействия; - 15 сут. воздействия.
1 – 0,5% ОМДА – 10%; 2 – 1,0% ОМДА – 10%; 3 – 2,0% ОМДА – 10%; 4 – 0,5% ОМДА – 25%; 5 – 1,0% ОМДА – 25%; 6 – 2,0% ОМДА – 25%; 7 – 0,5% ОМДА – 50%; 8 – 1,0% ОМДА – 50%; 9 – 2,0% ОМДА – 50%; 10 – ПГМГгх – 0,5%; 11 – ПГМГгх – 1%; 12 – ПГМГгх – 2,0%; 13 – Контроль

Samples of (co)polymers containing 30% OMDA (Samples 5-6) with a higher concentration in a 1-2% solution have the greatest biocidal effect, and that of Sample 9 is slightly less prominent. The weakest fungicidal properties were typical of the HMDA:OMDA:GHC (co)polymers with a ratio of 0.7:0.3:1.0 (Sample 4) and the PHMGhc (co)polymers (Sample 10) with a concentration of 0.5% in the solution. Wood samples treated with (co)polymers 5 and 6 at the initial stage of exposure were fully resilient against the contamination with *A. niger*, and the insignificant fungus growth on the surface (4.90 and 0.85% against the reference) was only observed at the end of the experiment. Other (co)polymer combinations had medium efficiency.

The correlation between the biocidal action and macromolecular design of (co)polymers aligns well with previously-obtained data. Thus, we showed that (co)polymers have similar impacts on both gram-positive and gram-negative bacteria [16, 10, 17]. The increase in the biocidal action against bacteria is associated with the increase in the hydrophobic fragments of the macromolecule, in particular the number of methylene groups, which results in increased absorption on the cell membrane.

The cell membrane is an important structure that is flexible and permeable. It maintains the integrity and vitality of microorganism cells, including fungus cells. It plays an important role in different biological functions, such as cell permeability control and protecting the cell from osmotic and mechanical impacts [18-22].

Cell membranes of fungi and bacteria differ by the type of polysaccharides: fungi contain chitin, a nitrogen-containing polysaccharide, while bacteria feature a unique polysaccharide comprising sugars and amino acids. For instance, the cell membrane of *A. niger* contains chitin and glucan [23]. The obtained similar correlations allow us to assume that the cell membrane interacts with (co)polymers in a similar way. Thus, the proteins that act as adhesins in cell membranes and allow the fungus cells to stick to the substrate [24-26] are responsible for the absorption of macromolecules by the fungus cell membrane during the first stage, after which the cell integrity is broke due to the appearance of a new material that can be connected to the ionic groups in mycelium walls [27]. As a result, the structural integrity and morphology of the cell deteriorate leading to the osmotic lysis [28]. Thus, we can claim that as the content of octamethylene fragments in the macromolecular structure increases, i.e. as the hydrophobic properties of (co)polymers increase, their fungicidal activity increases as well (Fig. 2). However, Sample 9 shows greater activity compared to Sample 12, which can be a consequence of the macromolecule hydrodynamic volume reduction due to the conformation mobility of a flexible structure [29] and result in the reduction of the contact area with the cell membrane.

Table 3

Efficiency classification in terms of protective ability [14]

***Таблица 3.* Классификация эффективности защитных средств по защищающей способности [14]**

Concentration, %	Average fungus contamination area, %	Antiseptic classification
Up to 3	0 – 10	High-efficiency
	10 – 30	Efficient
	30 – 50	Medium-efficiency
	Over 50	Inefficient

Table 4

The classification of PHMGhc and its copolymers in terms of their protective ability

***Таблица 4.* Классификация ПГМГгх и его сополимеров по защищающей способности**

Sample No	Antiseptic classification
1	Medium-efficiency
2	Medium-efficiency
3	Medium-efficiency
4	Efficient
5	High-efficiency
6	High-efficiency
7	Medium-efficiency
8	Medium-efficiency
9	Efficient
10	Medium-efficiency
11	Medium-efficiency
12	Efficient
13*	-

Notes: *Reference sample without (co)polymer treatment

Примечания: *Контрольный образец без обработки (co)полимером

Besides, the data obtained in the live experiment can be compared to the experiment conducted using the serial dilution method and they help us classify the (co)polymers depending on their protective properties against wood decay fungi. The classification parameters are shown in Table 3 [14].

Besides, the data obtained in the live experiment can be compared to the experiment conducted using the serial dilution method and they help us classify the (co)polymers depending on their protective properties against wood decay fungi. The classification parameters are shown in Table 3 [14].

According to the efficiency classification above, the (co)polymers in question belong to the medium-efficiency, efficient, or high-efficiency groups depending on their composition and concentration (Table 4).

CONCLUSIONS

1. We demonstrated the prominent fungicidal activity of water-soluble (co)polymers containing guanidines against *A. niger* using the serial dilution method and GOST 30028.4.-2006 Protective Solutions for Wood.

2. Thus, as the content of octamethylene fragments in the macromolecular structure increases, i.e. as the hydrophobic properties of (co)polymers increase, their fungicidal activity increases as well.

3. We established that the maximum suppression of fungal growth is achieved for the nutrient medium containing 30% and 50% of octamethylenediamine, which correlates with the data obtained in the assessment of the fungicidal action on the common pine wood. According to the efficiency classification, the compounds in question are highly-efficient in terms of wood protection properties.

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The authors declare the absence a conflict of interest warranting disclosure in this article.

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