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DISINFECTIVE ACTIVITY OF 8-HYDROXYQUINOLINE SULFATE ON MOULDS

AKTYWNOŚĆ DEZYNFEKCYJNA SIARCZANU 8-HYDROKSYCHINOLINY WZGLĘDEM GRZYBÓW PLEŚNIOWYCH

Summary: The aim of conducted research was the estimation of 8-hydroxyquinoline sulfate activity on selected mould strains. In the tests for the fungicidal activity, 6 working concentrations rangeing from 0.01 to 1.0% of 8-hydroxyquinoline sulfate were tested in terms of their efficiency. All strains under study were isolated from the building compartments and they were: *Cladosporium cladosporioides*, *Alternaria tenuissima*, *Stachybotrys chartarum*, *Aspergillus flavus* and *Penicillium notatum.* The effect of fungicide activity against moulds was assessed by means of diffusion cylinder-plate method. The rate of mycelial growth and the ability to germinate in the presence of the tested chemical was also estimated. In the cylinder-plate the biggest - 85.0 mm zone of growth inhibition was obtained at the concentration of 1.0% for *Aspergillus* and additionally for *Penicillium* at the concentration of 0.75%. The same sizes of the zones (85.0 mm) were noted for *Stachybotrys* at 0.5% concentration, and for *Cladosporium* and *Alternatia* at 0.25% concentration. On the basis of the growth rate of mycelium, it was noted that the most sensitive to 8-hydroxyquinoline sulfate activity are *Stachybotrys* and *Alternaria* strains. The lowest inhibitory dose which inhibited the mycelial growth completely was 0.2%. The effective activity of the examined chemical at following concentrations: 0.25; 0.5; 0.75; and 1% against the ability to germinate was observed for *Cladosporium*, *Stachybotrys* and *Alternaria*. The results obtained in the research showed that the inhibition of mycelium and spores were effective in case of 1% of 8-hydroxyquinoline sulfate.

Keywords: 8-hydroxyquinoline sulfate, moulds, disinfection

Moulds, when growing in buildings may cause biological corrosion and pose health hazard to people in the buildings. Biological corrosion of building materials, occuring due to very intensive surface development of moulds can worsen the aesthetic values of the building and may cause the loss of mechanical properties of infested elements. However, much worse than corrosion of materials is the influence of moulds on peoples' health. They have been reported [1] many times to be the reason for many diseases.

The basic method used when fighting against moulds on building materials is introducing chemicals with biocidal properties (fungicides), into materials during the production process or during the usage.

The aim of the research was the estimation of fungicidal activity of 8-hydroxyquinoline sulfate with reference to typical mycoflora occurring in buildings with biodeterioration evidence. The assay of moulds sensitivity was run in relation to mycelium and spores. Obtained results were used to analyse the abilities to apply the most effective concentrations of the chemical.

Materials and methods

Moulds under study belonged to most frequently isolated moulds from buildings with biodeterioration evidence [2], and based on performed identification they were categorized

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as: *Alternaria tenuissima*, *Aspergillus flavus*, *Cladosporium cladosporioides*, *Penicillium notatum*, *Stachybotrys chartarum.*

In the tests for fungicidal activity, the efficacy of 8-hydroxyquinoline sulfate was tested in 6 working concentrations of: 0.01, 0.1, 0.25, 0.5, 0.75 and 1.0%.

The fungicidal activity of 8-hydroxyquinoline sulfate was assessed on the basis of: the cylinder-plate diffusion method, the intensity of mycelial growth rate and the ability of spores to germinate.

Fungicidal activity of the chemical applied was assessed with the modified cylinder-plate diffusion method. The dishes were poured with 20 cm^3 of Sabourauda medium inoculated with particular moulds species and the mixture of the spores of the tested moulds (of 1×10^6 cell/cm^3 density). The wells were filled with 0.2 cm^3 of the tested chemical in respective concentrations. The control treatment was the wells filled with sterile water. The samples were incubated at 25^{o}C for 3 weeks. The disinfective efficacy of the tested chemical was assessed on the basis of the growth inhibition zones.

The indication of fungicidal activity based on the intensity of mycelial growth rate was conducted on Sabourauda medium with the addition of the consecutive concentrations of the chemical. Agar discs of 10 mm diameter overgrown with 2-week mycelium of tested moulds were placed on the medium on Petri dishes. The control treatment was prepared on a Petri dish with Sabourauda medium (without the chemical) and mycelium disc. The diameter of mould colonies was measured every 3 days, from the beginning of the mycelial growth recorded in the control dishes. The samples were incubated at 25°C.

The activity of tested chemical against the mycelial growth was assessed on the basis of the growth rate index (T), calculated with the following formula [3, 4]:

$$T = \frac{A}{D} + \frac{b_1}{d_1} + \ldots + \frac{b_x}{d_x}$$

where: T - growth rate index, A - the mean value of colony diameter measurement [mm], D - the length of an experiment [days], b_1, b_2, b_x - increase in diameter since the last measurement, d_1, d_2, d_x - number of days passed since the last measurement.

Indication of fungicidal activity was also tested on the basis of the spores' ability to germinate. For this purpose 0.02 cm^3 of the consecutive concetrations of 8-hydroxyquinoline sulfate were placed on the 1 cm^2 of the slide. After drying, preparations were inoculated with 0.02 cm^3 of the suspension containing spores of the tested moulds. The density of inoculum was selected in a way that in the field of vision under a medium magnification of a microscope 50÷60 spores were observed. The germination of 50 spores was assessed after 24 hours in the field of vision under a microscope. When rating germination, the scale from 0 to 4 introduced by Burgieł [3, 4] was used.

The influence of tested chemicals on the development of mould spores was assessed on the basis of the spores germination index, calculated with the formula:

$$I = \frac{\sum(n \times a) \times 100}{N \times 4}$$

where: I - spores germination index, n - number of the spores in the specific grade on the scale, a - grade on the scale, N - general number of the counted spores, 4 - the highest grade of the scale.

Results

8-hydroxyquinoline sulfate $(C_9H_7NO)_2 \cdot H_2SO_4$ is a chemical with known bacteriocidal and fungicidal properties, which has not been used so far against moulds growing on building materials.

Assay of biocidal activity of tested chemicals was based on the size of the growth inhibition zone, measured in mm. In case of 8-hydroxyquinoline sulfate the lowest concentration of the chemical was taken into account above which no significant differences were noted but the biggest growth inhibition zones were obtained. The biggest - 85.0 mm zones were obtained in the concentration of 1.0% for *Aspergillu*s and the mixed treatment and additionally for *Penicillium* in the concentration of 0.75%. The same sizes of the zones (85.0 mm) were noted for *Stachybotrys* in 0.5% concentration and for *Cladosporium* and *Alternaria* in 0.25% concentration. The increase in concentrations in all cases did not have an effect on the size of the zones and the differences between them were not significant (Table 1).

Table 1

Zones of the growth inhibition of tested moulds in the presence of 8-hydroxyquinoline sulfate

Mould strain	Control	Concentration of applied chemical [%]					
		0.01	0.1	0.25	0.50	0.75	1.0
Penicillium	10.00 a	10.00 a	10.00 a	60.83 b	70.00 c	85.00 d	85.00 d
Aspergillus	10.00 a	10.00 a	10.00 a	10.00 a	10.00 a	10.00 a	85.00 b
Cladosporium	10.00 a	10.00 a	16.33 b	85.00 c	85.00 c	85.00 c	85.00 c
Stachybotrys	10.00 a	10.00 a	10.00 a	10.00 a	85.00 b	85.00 b	85.00 b
Alternaria	10.00 a	10.00 a	10.00 a	85.00 b	85.00 b	85.00 b	85.00 b
Mixed treatment	10.00 a	10.00 a	10.00 a	10.00 a	10.00 a	10.00 a	85.00 b

lower case - significant differences ($p \leq 0.05$)

The values of inhibition zones were evaluated on the basis of obtained results and contributed to a specification of three mould groups according to their activity:
1st group - zone of the growth inhibition below 40 mm - resistant strain,
2nd group - zone of the growth inhibition 40÷60 mm - medium-sensitive strain,
3rd group - zone of the growth inhibition above 60 mm - sensitive strain.

In order to obtain the value of the inhibition zone above 40 mm, for all of the strains at the same time, the 8-hydroxyquinoline sulfate should be applied in the concetration of 1.0%.

The activity of 8-hydroxyquinoline sulfate on the linear growth of mycelium of tested moulds was determined on the basis of the growth rate index (GRI). In the conducted test, the diameter of the colonies [mm] growing on the media containing consecutive concentrations of the chemical was measured including the increase in the diameter at time intervals. The objective was to find the lowest concentration for which the GRI had low values, statistically significant in respect to the control treatments.

The most sensitive to 8-hydroxyquinoline sulfate were *Stachybotrys* and *Alternaria* strains. The lowest inhibitory dose which inhibited the mycelial growth completely was 0.25%. Slightly higher concentration - 0.5% inhibited *Cladosporium*, and the next one - 0.75% was the lowest inhibitory concentration to *Aspergillus*. In case of *Penicillium* and the mixed treatment, the concentration which inhibited the growth was 1%. For all other

concentrations, the growth of tested moulds was not observed above the lowest inhibitory value (GRI = 0) (Table 2).

Table 2

The growth rate index of tested moulds in the presence of 8-hydroxyquinoline sulfate

Mould strain	Control	Concentration of applied chemical [%]					
		0.01	0.1	0.25	0.50	0.75	1.0
Penicillium	25.35 c	23.29 c	23.87 c	23.96 c	24.29 c	20.42 b	0 a
Aspergillus	16.03 e	15.40 e	13.23 d	10.52 c	2.29 b	0 a	0 a
Cladosporium	15.60 e	14.97 d	8.31 c	5.58 b	0 a	0 a	0 a
Stachybotrys	24.37 d	22.70 c	7.23 b	0 a	0 a	0 a	0 a
Alternaria	33.05 d	32.06 c	29.97 b	0 a	0 a	0 a	0 a
Mixed treatment	25.95 e	22.37 c	24.37 d	23.20 c	22.46 c	20.42 b	0 a

lower case - significant differences ($p \leq 0.05$)

In the laboratory research the influence of 8-hydroxyquinoline sulfate on the germination of tested mould spores was estimated. The influence of the chemical under study on the development of spores was estimated on the basis of the microscopic observations, which also enabled determination of the germination index (GI), taking into account the grade of the hyphae germination on the scale 0-4.

For the following moulds: *Penicillium*, *Cladosporium* and *Stachybotrys* the spores' germination was inhibited starting from the concentration of 0.5%. There were no significant differences between the consecutive concentrations: 0.5; 0.75 and 1%. For *Aspergillus* only 1% concentration inhibited effectively spores germination. The value of GI was 0 similarly to the values for *Cladosporium* and *Stachybotrys*. It was also observed that in case of *Alternaria* there were no statistically significant differences between the concentrations (from 0.1 do 1%). The lowest value of GI was obtained when applying 1% dose (GI = 0.66%) (Table 3).

Table 3

The ability of the spores to germinate in the presence of 8-hydroxyquinoline sulfate

Mould strain	Control	Concentration of applied chemical [%]					
		0.01	0.1	0.25	0.5	0.75	1.0
Penicillium	24.50 e	14.33 d	7.16 c	4.50 b	1.83 a	0.66 a	0.33 a
Aspergillus	20.50 f	12.50 e	7.16 d	4.33 c	1.16 b	0.33 ab	0 a
Cladosporium	35.33 d	23.16 c	4.00 b	2.83 ab	0.83 a	0.50 a	0 a
Stachybotrys	74.50 d	32.66 c	6.16 b	4.33 ab	1.33 a	0.33 a	0 a
Alternaria	81.33 c	24.33 b	4.50 a	2.50 a	1.66 a	1.16 a	0.66 a

lower case - significant differences ($p \leq 0.05$)

The activity of biocidal chemicals against the microorganisms is a complex and multi-stage reaction, which mechanism has not been fully known. Therefore it is difficult to predict the effect on the moulds of different fungicidal environment.

The conducted research enables to conclude that only on the basis of obtained results concerning the growth of vegetative mycelium and the reaction of the spores towards the specific chemical, it is possible to determine its fungicidal activity. In the studies only

8-hydroxyquinoline sulfate in the concentration of 1% was inhibitory for both the mycelium and the spores.

Summary and conclusion

The research proved a considerable diversity of sensitivity among particular mould strains to particular concentrations of 8-hydroxyquinoline sulfate included in tests and enabled to draw the following conclusions:

1. An impact of the chemical on the linear growth of mycelium can be determined on the basis of the growth inhibition zones and the growth rate index. The effective concentration of the chemical is the one which causes the inhibition zone bigger than 60 mm or inhibits the mycelium growth completely.
2. On the basis of performed analysis it was concluded that heterocyclical chemicals containing N display high fungicidal activity. Application of 8-hydroxyquioline sulfate inhibits the mycelial growth and the spores' germination of the moulds.
3. The sensitivity of moulds to fungicide is a species trade. The most sensitive to 8-hydroxyquinoline sulfate were the following strains: *Cladosporium cladosporioides*, *Stachybotrys chartarum* and *Alternaria tenuissima*, and the most resistant strains were: *Aspergillus flavus* and *Penicillium notatum*.

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AKTYWNOŚĆ DEZYNFEKCYJNA SIARCZANU 8-HYDROKSYCHINOLINY WZGLĘDEM GRZYBÓW PLEŚNIOWYCH

Streszczenie: W pracy przeprowadzono badania mające na celu ocenę działania siarczanu 8-hydroksychinoliny na wybrane szczepy grzybów strzępkowych. W testach aktywności grzybobójczej sprawdzono działanie siarczanu 8-hydroksychinoliny o 6 stężeniach roboczych od 0,01 do 1,0%. Grzybami testowymi były gatunki wyizolowane z przegród budowlanych, a mianowicie: *Cladosporium cladosporioides*, *Alternaria tenuissima*, *Stachybotrys chartarum*, *Aspergillus flavus* oraz *Penicillium notatum*. Efekt biobójczego działania związku na grzyby pleśniowe oceniono metodą dyfuzyjną płytkowo-cylinderkową. Przeprowadzono również ocenę tempa wzrostu grzybni oraz zdolność kiełkowania zarodników grzybów pleśniowych w obecności testowanego związku. W metodzie dyfuzyjnej płytkowo-cylinderkowej największe - 85,0 mm strefy zahamowania wzrostu uzyskano w roztworze o stężeniu 1,0% dla *Aspergillus* oraz dodatkowo dla *Penicillium* w roztworze o stężeniu 0,75%. Takie same wartości stref (85,0 mm) uzyskano dla *Stachybotrys* przy 0,5% stężeniu, a dla *Cladosporium* i *Alternaria* przy 0,25%. Na podstawie oceny tempa wzrostu grzybni stwierdzono, iż najbardziej wrażliwe na działanie siarczanu 8-hydroksychinoliny są szczepy *Stachybotrys* oraz *Alternaria*. Najmniejsza dawka hamująca całkowicie wzrost grzybni to 0,2%. Ponadto zaobserwowano skuteczne działanie 0,25; 0,5; 0,75 i 1% badanego związku na zdolność kiełkowania zarodników *Cladosporium*, *Stachybotrys* i *Alternaria*. Z przeprowadzonych badań wynika, że inhibicję zarówno w stosunku do grzybni, jak i zarodników wykazał 1% siarczan 8-hydroksychinoliny.

Słowa kluczowe: siarczan 8-hydroksychinoliny, pleśnie, dezynfekcja