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Małgorzata NABRDALIK[1]

DISINFECTIVE ACTIVITY OF 8-HYDROXYQUINOLINE SULFATE ON MOULDS

AKTYWNOŚĆ DEZYNFEKCYJNA SIARCZANU 8-HYDROKSYCHINOLINY WZGLĘDEM GRZYBÓW PLEŚNIOWYCH

Abstract: The aim of conducted research was the estimation of 8-hydroxyquinoline sulfate activity on selected mould strains isolated from the buildings with biodeterioration evidence. 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 method the criterion for assessing fungicidal activity was based on the size of the zone of growth inhibition (measured in mm). 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. The increase in concentrations in all cases did not have an effect on the size of the zones of growth inhibition. 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

Filamentous fungi, commonly called 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 [1]. However, much worse than corrosion of materials is the influence of moulds on peoples' health. They have been reported [2–4]

[1] Department of Biotechnology and Molecular Biology, Opole University, ul. kard. Kominka 4, 45–035 Opole, Poland, phone: +48 77 401 60 56, email: mnabrdalik@uni.opole.pl

many times to be the reason for many diseases. Some of the moulds are allergic for people with a healthy immune system. However, they may damage the neural system, liver, kidneys or cause changes leading to cancer diseases of people with immune deficiency. Besides, moulds have specific biological properties and biochemical abilities enabling them to produce many toxic metabolites, including the most dangerous ones – mycotoxins (aflatoxin, ochratoxin) [5, 6].

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 efficacy of chemical methods for the protection of building materials against moulds depends on fungicidal activity of applied chemical and the type of the moulds. Therefore, tested moulds should be representative for the environment, in which disinfection will be carried out. It cannot be one dominant species but the mixture of several strains, as in natural environment we deal with mixed mycoflora.

The aim of the research was the estimation of fungicidal activity of 8-hydroxyquinoline sulfate with reference to typical mycoflora occuring in buildings with biodeterioration evidence. The assay of moulds sensitivity was run in relation to mycelium and spores. Obtained reults 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 [7], and based on performed identification they were categorized as:

– *Alternaria tenuissima* (Fries) Wiltshire,
– *Aspergillus flavus* Link,
– *Cladosporium cladosporioides* (Fresenius) de Vries,
– *Penicillium notatum* Westling,
– *Stachybotrys chartarum* (Ehrenberg ex Link) Huges.

The strains were kept on agar slant tubes containing 10 cm^3 of Sabourauda medium. They were incubated at 25 ^oC for 14 days. After this period, the sporulating mycelium was washed three times with the isotonic solution of sodium chloride. The density of material, measured with a counting chamber Thoma type, was stable and equal to $1 \cdot 10^6$ cells/1 cm^3.

For further studies the suspension of particular moulds species was used as well as the mixture of the moulds spores in proportion (1 : 1 : 1 : 1 : 1).

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,
– the ability of spores to germinate.

Fungicidal activity of the chemical applied was assessed with the modified cylinder-plate diffiusion method where 3 sterile glass cylinders of 10 mm diameter and 12 mm of height were placed on Petri dishes (∅ 100 mm). Next, 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 $1 \cdot 10^6$ cell/cm^3). During the medium inoculation its temperature did not exceed 50 °C. The plates with agar were then left at room temperature to solidify. After removing the cylinders, 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 tests were conducted in six replicates (A, B, C, D, E, F), where each well was a single treatment. The samples were incubated at 25 ^oC for 3 weeks, and the measurements were taken after 3, 7, 10, 14 and 21 days. The disinfective efficacy of the tested chemical was assessed on the basis of the growth inhibition zones measurement made exact to 0.1 mm. In replicates, where moulds overgrew the medium around the wells completely, the value of the measurement was assumed 10 mm. In cases when the mycelium growth was not noted the measurement value was assumed 85 mm.

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 exact to 0.1 mm, from the beginning of the mycelial growth recorded in the control dishes. The measurements were recorded until the mould in the control treatment reached the edge of the dish. The test was conducted in four replicates (A, B, C, D), while one Petri dish with the mycelial disc represented a single replica. 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 [8, 9]:

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

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.

Test results were calculated as the percentage of the growth inhibition.

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 slides with the drops of suspension were placed in moist chambers. The chambers were prepared as Petri dishes with blotting paper discs on the bottom, saturated with sterile water. The control treatment was pure spores suspension placed on a clean slide. The test was conducted in four replicates (A, B, C, D). The germination of 50 spores was assessed after 24 hours in the field of vision under a microscope. When rating germination, the scale introduced by Burgiel [8, 9] and Kowalik [10] was used:

0 – non-germinating conidia,
1 – the length of a germ tube shorter than the spore length,
2 – the length of a germ tube equal to the length of a spore,
3 – the length of a germ tube twice as long as the spore length,
4 – branching germ tube repeatedly longer than a conidium.

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:

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

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.

The efficacy of the fungicide was assessed as the % of the spores development inhibition.

Results

8-hydroxyquinoline sulfate $(C_9H_7NO)_2 \cdot H_2SO_4$ is a chemical with known bactericidal 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 %. However, for *Penicillium, Cladosporium* and *Alternaria* the lowest inhibitory concentration was 0.25 % and for *Stachybotrys* 0.5 %. 8-hydroxyquinoline sulfate has a fungicidal effect against all tested mould strains and their spores mixture in the concentration of 1.0 % and the measured inhibition zones were the biggest – equal to 85.0 mm.

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). The growth of mycelium of all tested moulds was inhibited in 100 % at 1 % concentration of the sulfate (Fig. 1). Furthermore, the solutions of 8-hydroxyquinoline sulfate did not affect the morphology of the colony only brighter pigmentation of an aerial mycelium was noted. The structure of the colonies was not changed. The production of the spores was inhibited in all combinations.

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 ($p \leq 0.05$).

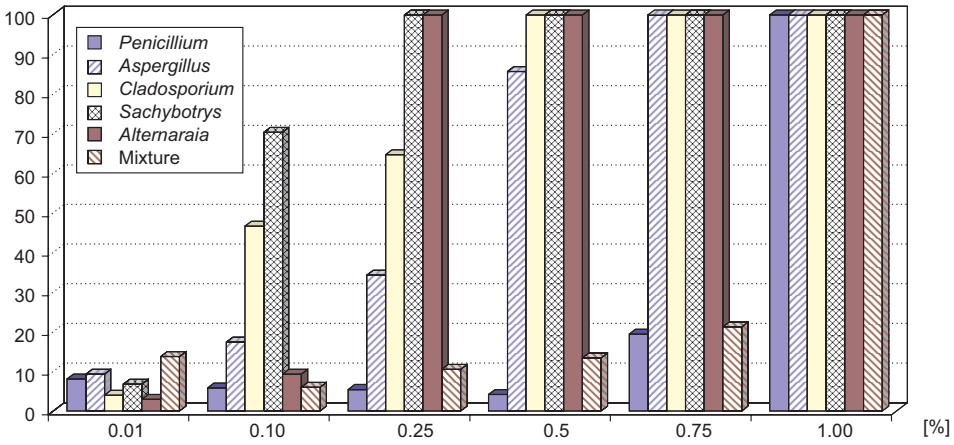

Fig. 1. The decrease in the growth rate of tested moulds after application of 8-hydroxyquinoline sulfate

In the fight against moulds the desired effect of a fungicide application is the complete inhibition of the mycelial growth, therefore the concentrations considered fungicial are only those in which 100 % of efficacy was obtained against all tested moulds and the mixture of their spores. The condition was fulfilled only by 1 % 8-hydroxyquinoline sulfate (Fig. 1). The aforementioned concentration will be treated at the same time as a minimal concentration inhibiting the development of mould mycelium.

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 ($p \leq 0.05$).

Also the highest reduction in the number of spores ranging from 97.34 to 100 % was obtained in the concentration of 1 %. (Fig. 2).

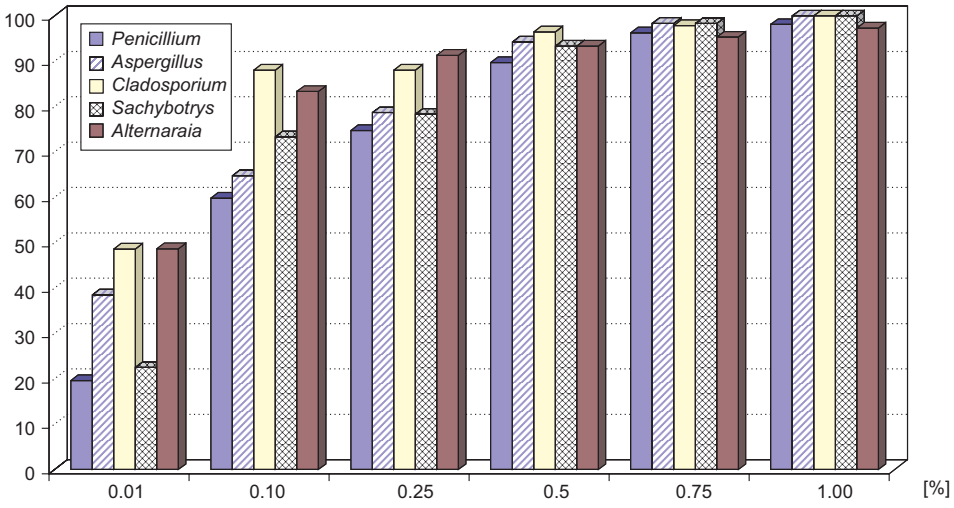

Fig. 2. The reduction of germinating spores after application of 8-hydroxyquinoline sulfate [%]

During the microscopic observation it was noted that the chemical did not affect the morphology of the spores of tested moulds. The changes in the size, colour and external structure of the spores were not observed.

When assessing the toxicity of fungicides the scale introduced by Kowalik and Krechniak [10] can be used:

0–49 % – ineffective chemical,

50–79 % – sufficiently effective chemical,

80–90 % – efficient chemical,

91–100 % – very efficient chemical.

Based on the scale, it was established, that the fungicide is biologically active if its toxicity is over 50 % [8]. However, in the presented study the concentration of the chemical was regarded as fungicidal, for which the effectiveness towards the spores was 90 %. The above assumption was true for the spores of all tested strains only in case of 8-hydroxyquinoline sulfate in the concentrations of 0.75 and 1 %.

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

The conducted research enables to state 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. The sensitivity of the spores should be determined on the basis of a germination ability in the presence of the chemical. The effective chemical is characterized by over 90 % of efficacy.

3. The conducted tests prove that biological chemicals display a varied biocidal activity against mycelium and spores. The fungicidal effect was stronger in case of the spores rather than the mycelium. Therefore, the estimation of fungicidal activity should be conducted on the basis of sensitivity to the vegetative mycelium and mould spores.

4. On the basis of performed analysis it was stated 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.

5. 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*.

6. The estimation of fungicial activity should be conducted on moulds which are representative for the environment, in which disinfection will be carried out. It should not be one dominant species, but the mixture of several strains.

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AKTYWNOŚĆ DEZYNFEKCYJNA SIARCZANU 8-HYDROKSYCHINOLINY WZGLĘDEM GRZYBÓW PLEŚNIOWYCH

Samodzielna Katedra Biotechnologii i Biologii Molekularnej
Uniwersytet Opolski

Abstrakt: W niniejszej pracy przeprowadzono badania mające na celu ocenę działania siarczanu 8-hydroksychinoliny na wybrane szczepy grzybów strzępkowych wyizolowanych z budynków z oznakami biodeterioracji pleśniowej. W testach aktywności grzybobójczej sprawdzono działanie siarczanu 8-hydroksychinoliny w 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 podstawą do określenia aktywności biobójczej były rozmiary strefy zahamowania wzrostu [mm]. Największe – 85,0 mm strefy zahamowania wzrostu uzyskano dla stężenia 1,0 % dla *Aspergillu*s oraz dodatkowo dla *Penicillium* w 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 %. Wzrost o kolejne stężenia we wszystkich przypadkach nie miał wpływu na wartości stref zahamowania wzrostu. Na podstawie oceny tempa wzrostu grzybni stwierdzono, iż najbardziej wrażliwe na działanie siarczanu 8-hydroksychinoliny są szczepy *Stachybotrys* oraz *Alternaria*. Najniższa 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