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Conference Paper *in* AIP Conference Proceedings · November 2017

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Citation: AIP Conference Proceedings **1899**, 050006 (2017);
View online: https://doi.org/10.1063/1.5009869
View Table of Contents: http://aip.scitation.org/toc/apc/1899/1
Published by the American Institute of Physics

Comparative Evaluation of the Activity of Commercial Biocides in Relation to Micromycetes

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Abstract. The paper presents the results of comparative studies of commercial biocides of Russian production, used in the technology of biostable materials for construction purposes, to micromycetes of various species and type diversity. The influence of the type of biocide additive on their fungicidal activity was established. The fungicidal effect of bioactive agents was evaluated using a disc-diffusion method. The analysis of the results is carried out both using the traditional approach and a modified method using a scoring of the degree of impact.

INTRODUCTION

Lower organisms live permanently all over the environment of human presence, using organic and inorganic compounds as a nutrient substrate [1−3]. Biological contamination of structures and buildings leads to a violation of the sustainable state of the environment, as a result of which there is an increase in diversity and abundance, including pathogenic microorganisms [4]. The demanded intensification of agricultural development, focused on the production of food products of plant and animal origin, as well as the development of alternative energy technologies, including those obtained using biotechnology, brings the outlined problems to a new level.

One of the most effective and long-term surface methods of protecting building materials and structures from damage by microorganisms is the use of biocidal agents [5, 6]. According to the report [7], the world market of chemicals as of 2016 is characterized by a profit of 9.4 billion. The leader in the production of biocides is North America. The rapid development of China and India, and the demand for agents in them, predicts the growth of the market in the Asia-Pacific direction, which is due, first of all, to a progressive increase in the number of strains resistant to them. Nevertheless, the resistance of pathogenic microorganisms poses a serious problem and directly affects the effectiveness of sanitary and hygienic measures. It should be noted that active substances, the use of which is conditioned by the need to prevent biodegradation or eliminate the effects of the destructive effect of the technophile biodestructor, as a rule, have a complex effect on the formed microbiocenosis in the aggregate. The choice of biocidal preparations used in each specific case depends on the prevailing types of microorganisms in the composition of biota, attacking materials, structures or constructions in general, the conditions of use, the chemical properties of active substances, their environmental safety, etc. [8−10].

The goal of the presented work is a comparative analysis of the activity of agents entering into different groups according to the type of action differing in the chemical basis of the active components in relation to the most common mycocenosis destructors attacking the surface of building materials in the conditions of a modern man-made environment.

Prospects of Fundamental Sciences Development (PFSD-2017)
AIP Conf. Proc. 1899, 050006-1–050006-5; https://doi.org/10.1063/1.5009869
Published by AIP Publishing. 978-0-7354-1587-4/$30.00

EXPERIMENTAL PART

Materials

The following bioactive substances were chosen for testing: the disinfectant “Dinovis” manufactured by closed joint stock company “Aldomed” (Tomsk, Russia) [11] and the bactericidal additive “BioPlast” [12] produced by limited liability company “Polyplast Novomoskovsk” (Novomoskovsk, Russia).

“Dinovis” is a formaldehyde-free agent for disinfecting veterinary surveillance objects and prevention of animal infectious diseases. It is an aqueous concentrate containing glyoxal, alkyldimethylbenzylammonium chloride (C12−C14), 2-methyl imidazole and functional components.

“BioPlast” is used as a bactericide in order to prevent biodegradation and mold formation at already finished constructions. It is an aqueous solution of bactericidal materials containing a composition of two different bactericidal substances, which differ in the nature of the active effect on microorganisms: a modified mixture of polyhexamethylene guanidine hydrochloride and quaternary ammonium salts.

The choice of additives is due to the results of preliminary studies performed by the team: biocidal additives were introduced into the composition of building materials of various compositions to ensure their biostability while maintaining the required physico-mechanical characteristics. The use of disinfectants in low concentrations is the first factor in the formation of the stability of bioagents. Most manufacturers intentionally indicate low working concentrations for their attractiveness in the economic aspect, giving efficiency a secondary importance. In this basis the recommended by the manufacturer minimum and maximum concentrations were used as sorted out: for Dinovis (0.5%, 5%), for BioPlast (0.025%, 0.25%).

Despite the fact that the main prevailing active substances of the used commercial biocidal agents have been separately studied for resistance to micromycetes by a number of authors [13−17], in each individual case it is necessary to investigate the joint action of biocidal components on a particular type of microorganism.

Previously, we conducted researches to study the mycobiota of surface areas of buildings with characteristic signs of mold damage, which resulted in the identification of the most prevalent fungal genera [18]: *Aspergillus niger van Tieghem, Aspergillus terreus Thorn, Penicillium cyclopium Westllng, Penicillium purpureum, Chaetomium globosum Kunze, Paecilomyces varioti Bainier.* Pure test cultures of the detected microscopic fungi were obtained from the laboratory of biochemistry of fungi of the Botanical Institute named after V.L. Komarov of Russian Academy of Sciences (Russia).

Research Methods

The activity of biocides against monocultures was evaluated by the disco-diffusion method [19, 20]. This technique is regulated in bacteriological studies, in particular, to study the sensitivity of microorganisms to antibiotic substances. In this work, a well-known research methodology was used, which was subsequently adjusted in accordance with the goal. The essence of the methodic used is in measuring the zone diameter of inhibition of monoculture growth, occurring around the paper disc, impregnated with fungicidal composition.

As a solid nutrient medium Czapek DOX was used, obtained by co-brewing of the following components: potassium monophosphate – 0.7 g; dipotassium hydrogen phosphate 3-hydrate – 0.3 g; magnesium sulfate 7-water – 0.5 g; sodium nitrate – 2.0 g; potassium chloride – 0.5 g; Ferrous (II) sulphate 7-aqueous – 0.01 g; saccharose – 9 g; distilled water – 1000 ml, agar – 20 g. The obtained medium was poured into sterilized Petri dishes, in the amount of 20 ml per cup, and dried for one hour.

Preparation of spore suspension of fungi was carried out in a following way: with the help of microbiological nichrome loops, the transfer of dry ripe spores of mold fungus monoculture was carried out. Spores were placed in a flask containing 50 cm^3 of sterile tap water, thoroughly mixed by shaking and rubbing with a glass rod with a rubber tip to prevent clumping. The obtained suspension was filtered through four layers of sterile gauze. The concentration of spores was counted using a Gorjaev’s chamber.

After the medium’s solidification the spore suspension of 10^6 CFU per 1 ml was applied in the amount of 0.2 ml per Petri dish and evenly distributed with a Dergalskiy’s spreader along the surface. When the medium congealed, the paper discs were placed on its surface at an equal distance from each other and at a distance of 1.5 cm from the edge of the dish. Each dish contained five disks: one of them was a control sample, and also four discs impregnated with each of the selected biocides in two different concentrations.

Discs of 8 mm diameter were made from filter paper of filtering density 65g/m^2, thickness 0.15 mm, the filtration capacity – no more than 15 seconds. The discs were impregnated with biocidal compounds’ solutions of selected concentrations. The disc impregnated with distilled water was taken as a control sample. The disc impregnation was carried out by single dipping them in the test solutions, then the discs were dried for 2 hours normal circumstances.

Petri dishes in an inverted state were incubated for seven days before the appearance of visual growth at a temperature of 23–25 ° C.

The experiment was carried out in a laminar flow hood. During the entire experiment the sterility of all used surfaces and labour dish was thoroughly monitored to prevent the ingress of microorganisms from the external environment.

The fungicidal activity of the agent applied to a paper disc was assessed by the growth pattern and the growth inhibition zone (mm), as well as the degree of fouling of the treated disc on the 5-point scale according to the procedure described in [21]:

0 – complete suppression of growth, formation of a zone of suppression of growth;
1 – total suppression of growth ;
2 – cobwebby mycelium;
3 – suppressed growth of mycelium;
4 – limited growth of mycelium, suppressed sporulation;
5 – abundant growth of mycelium, sporulation.

The presence and nature of the development of mycelial fungi was monitored using a microscope. The diameter of the test cultures growing zone inhibition was measured using a trammel. For the reliability of the results, the bottom of each Petri dish was pre-illuminated for the most accurate detection of fungicidal zones.

RESULTS AND DISCUSSION

The results of experimental studies on the fungicidal effect of the commercial agents used are presented in Tables 1 and 2.

The absence of cultures on the paper discs treated with the agents and, in a number of cases, directly around them, indicates a high fungicidal activity of the test materials; the appearance of individual colonies – indicates some overwhelming action; significant fouling of the discs – indicates the absence of any effect of the agent on the growth of the test culture.

TABLE 1. Effect of biocidal agents on the growth of test cultures.

Test cultures	Degree of treated discs fouling, points			
	Dinovis		BioPlast	
	0.5 %	5 %	0.025 %	0.25 %
Aspergillus niger	0	0	5	5
Aspergillus terreus	0	0	3	3
Chaetomium globosum	0	0	4	3
Paecilomyces varioti	0	0	3	3
Penicillium cyclopium	0	0	3	0
Penicillium purpureum	4	4	4	4

The data in Table 1 show that the Dinovis at recommended concentration (5 % solution) has a high fungicidal activity against five of the six test cultures: *Aspergillus niger, Aspergillus terreus, Chaetomium globosum, Paecilomyces varioti, Penicillium cyclopium*, whose growth was suppressed completely. With the 10 times reduction of Dinovis concentration (0.5%), the effect of suppression of fungal growth is preserved. Bioplast solutions of given concentrations do not show activity against the fungus *Aspergillus niger*. With respect to other microscopic fungi, this additive has a weak suppressive effect. In general, fungi of the *Penicillium* genus are the most vulnerable towards the "Bioplast"'s effect in comparison, for example, with the *Aspergillus* genus.

After establishing the fact of the sensitivity of the fungi to the preparations, a quantitative evaluation of their fungicidal properties with respect to various representatives of microscopic fungi was carried out in terms of the retardation in the growth of the fungi in direct contact conditions (Table 2).

The results of the experiment have been shown that the most sensitive to the Dinovis compound are the following micromycetes: *Penicillium cyclopium*, *Chaetomium globosum*, *Aspergillus niger*. Area of fungi growth absence is 5–9 mm. *Paecilomyces varioti* is the least sensitive towards this agent (2 mm), maximum biocide concentration has only a minimal growth inhibition, and in case of *Penicillium purpureum* the fungicidal activity is absent.

In case of Bioplast, the compound has a minimal impact on the fungi growth regardless of concentration and has no fungicidal activity. It should be noted that there is fungicidal effect of this biocide only towards *Penicillium cyclopium* (3 mm). It was mentioned that *Penicillium purpureum* was the most resistant towards the studied compounds regardless of the concentration, the inhibition zone growth was 0 mm.

TABLE 2. Evaluation of the biocidal agents fungicidal properties

Test cultures	**Zones of growth retardation, mm**			
	Dinovis		**BioPlast**	
	0.5 %	**5 %**	**0.025 %**	**0.25 %**
Aspergillus niger	3	5	0	0
Aspergillus terreus	2	4	0	0
Chaetomium globosum	3	6	0	0
Paecilomyces varioti	3	2	0	0
Penicillium cyclopium	7	9	0	3
Penicillium purpureum	0	0	0	0

Thus, the evaluation of the fungicidal properties of the two biocidal agents showed a different degree of suppression effect on fungal test cultures, with the apparent superiority of the agent "Dinovis".

The advantages of the agent "Dinovis" discovered during the course of the experimental studies can probably be explained by the fact that the preparation is a synergetic complex of several active components (aldehydes, quaternary ammonium compounds, imidazole derivatives) possessing various mechanisms of antimicrobial action, which provides a wider spectrum of antifungal activity.

CONCLUSIONS

The creation of biostable building materials allows to prevent growth of pathogens, biodegradation and ensure sanitary and hygienic conditions.

Mold fungi have a high adaptive ability to changes in environmental factors and, in particular, to biocidal preparations. Therefore, the search for new effective and less toxic biocides, the study of the action of various compositions of known agents, the development of new methods of antimicrobial protection will be constantly in demand.

Within the framework of the present study, the stability of microscopic fungi was evaluated on the basis of data on the effect of biocidal components of different effects on certain cultures of fungi. In real environmental conditions, biocenoses, occupying surfaces and penetrating into the microporous space and, thus, infecting the material in volume, are a combination of a huge number of representatives of microorganisms reacting differently to biocides. This significantly complicates the evaluation of the quality of substances active against organisms-destructors. One of the tasks of further research will be an analysis of the degree of exposure of the widely-proposed construction biocides today on the stability of "wild" mycocenoses, obtained from real environmental conditions.

Thus, as a result of the conducted studies, it was shown that the agent "Dinovis" has a broader spectrum of biocidal effect on the examined mold fungi than the agent "BioPlast". Dinovis shows maximum antifungal activity against *Penicillium cyclopium, Chaetomium globosum, Aspergillus niger* monocultures. It was also proved that the Bioplast has no acute fungicidal action, as a zone of inhibition of 3 mm diameter was not recorded in any case except the fungi of the *Penicillium cyclopium* genus. None of the selected compounds showed the activity towaeds the *Penicillium purpureum* genus. At the same time, the absence of dose-dependence of the fungicidal activity of the preparations was established: regardless of the concentration of the additives, the nature of the fungicidal effect persisted. The carried out researches indicate its greater efficiency when used as a fungicidal additive taking into account its introduction into the composition of materials for their protection from biodamage. In addition, the use of "Dinovis", which is an initial disinfectant, will ensure the complex microbiological resistance of composites while maintaining the required sanitary and bacteriological conditions.

ACKNOWLEDGMENTS

This work was supported by Russian Science Foundation (project number 7.872.2017/4.6).

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