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Evaluation of fungicidity of disinfectants according to the radial growth rate of test cultures on solid nutrients

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Abstract. The paper presents the results of the study of the effectiveness of the effect of some disinfectants on mold mushrooms-destructors of building materials, previously found on the surface of walls and floors of livestock complexes and farms, namely: *Aspergillus niger*, *Aspergillus sp.*, *Penicillium sp.*, *Cladosporium sp.*, *Mucor spp.* Different sensitivity of these micromycetes to the disinfectant used has been established, as both drugs have a fungicidal effect on all used test cultures in concentrations of 0.1% and 0.2%.

1. Introduction

Agro-industrial enterprises are most exposed to microflora of different species. It is connected with specificity of operation of buildings and constructions in conditions of high humidity, temperature, and influence of wastes. To ensure the required level of cleanliness of the premises, disinfection measures using active ingredients, including fungicidal effects, are required to prevent the development of micelial fungi on the surfaces of buildings.

Today there is a big problem in the field of synthesis of active drugs in relation to various test-cultures. This is primarily due to the changing climate and the increasing needs of the population, which in turn leads to the formation of new resistant strains and resistances of existing ones. The consequences of this process are clearly reflected in the operational condition of buildings and structures [1-5], located in the most aggressive environments - industrial areas.

Chemical synthesis plants offer a wide range of biocides that differ not only in their composition, but also in their mechanisms of influence on organisms. The main consumers of biocides remain the production of paints and varnishes, pharmaceuticals, as well as medical and construction industries. It is worth noting the active growth of research in the field of increasing the durability of building composites by applying active components [6, 7] or introducing them into the material [8-12].

When selecting a disinfectant, it is necessary to take into account its activity with respect to micromycetes, as well as the degree of their toxic effects on living organisms that fall into the affected area during treatment. In this regard, it is necessary to establish a minimum concentration of biocide, providing the required destructive effect on pathogens.

Currently, there is a great variety of methods for analyzing the fungicidal properties of active drugs, but a significant part of them is related to the subjective visual assessment of mushroom growth and development in the presence of biocides. However, a quantitative assessment of growth suppression can be considered more objective. In this regard, the purpose of this study was to assess the influence of Russian disinfectants on the development of micromycetes in direct contact with the radial growth rate



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of colonies on solid nutrients, which characterizes the degree of their fungicidity.

2. Materials and methods

Disinfectants Dinovis manufactured by CJSC "Aldomed" (Tomsk) [14] and Megadez manufactured by CJSC "SEZ VladMiVa" (Moscow) [15] were used as the most effective disinfectants, which was established on the basis of multi-criteria evaluation of biocide quality of different composition and functional purpose according to the degree of fungicidal activity of fungicidal fungicide [13].

Calculation of biocidal dosages was carried out taking into account their subsequent use in cement systems. Dosages of 0.03 % (corresponds to 0.2 % in the cement system), 0.06 % (corresponds to 0.4 % in the cement system), 0.1 % (corresponds to 0.66 % in the cement system) and 0.2 % (corresponds to 1.3 % in the cement system) were chosen as the bioactive drugs' tested concentrations. It should be noted that the investigational preparations are intended for treatment of different surfaces and have not been used in the composition of binders so far.

As test objects, we used micromycheate test-cultures selected in the course of previous studies [16], which populate the surface of construction structures (walls and floors) operated under real conditions of the largest agricultural objects of cattle breeding, pig breeding and poultry farming (chickens and turkey) in the Belgorod region: *Aspergillus niger*, *Aspergillus spp.*, *Penicillium spp.*, *Cladosporium spp.*, *Mucor spp.*.

The degree of influence of drugs on the development of micromychetes was estimated at direct contact of the drug with mushroom test-cultures by the radial growth rate of colonies in a dense nutrient environment.

For this purpose, the sterile Petri dishes were used to pour out the agarized nutrient medium of Chapek containing different amounts of disinfectants tested. After the environment hardened in sterile conditions with an injection into the center of the Petri dish, test-culture spores were introduced and the linear growth (diameter) of mushroom colonies was measured for 10 days. The control was a variant of mushroom growth in Petri dishes on a standard agarized environment of Chapek without the addition of test preparations. The diameter of the colonies was measured in two orthogonal to each other in three repetitions at certain intervals (once a day).

The Abbott equation [17] was used to quantify the degree of deceleration of radial growth of colonies:

$$T = [(D_k - D_o) / D_k] \times 100 \%, \quad (1)$$

where D_k is the diameter of the colony in control; D_o is the diameter of the colony in the experiment; T is the deceleration (in percent) of the radial growth of micromychet colonies when adding substances that suppress growth to the nutrient environment.

The linear colony growth rate deceleration factor is a reliable quantitative indicator for the determination of biocidal activity of various substances. This method eliminates the "human factor" because the evaluation of fungicidal activity is mathematically calculated and visually very clear.

3. Results

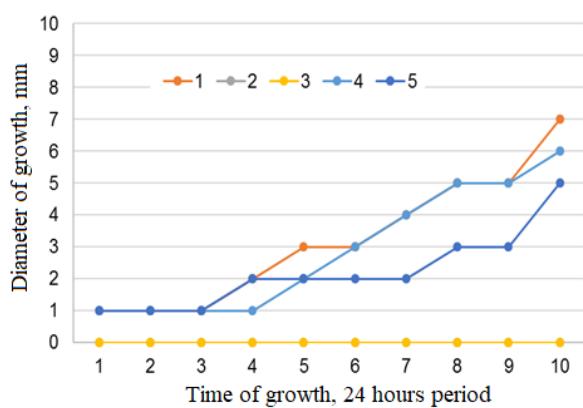
Visual examination of the cups on which microscopic fungi were cultivated, after the end of the experiment (10 days) indicated the complete absence of growth of *Aspergillus niger*, *Aspergillus spp.*, *Penicillium spp.*, *Cladosporium spp.*, *Mercury spp.* in variants with concentrations of Dinovis and Megadez 0.1% and 0.2% Table 1.

The growth of mushrooms in the control (clean) environment is characterized by their intensive distribution throughout the volume of the bowls with filling the free space of Figure 1. The minimum distribution diameter is *Aspergillus spp.* and the maximum is *Cladosporium spp.*

Introduction of Dynovis even in minimal concentration (0.03%) leads to absolute suppression of growth of *Penicillium spp.* and *Aspergillus niger* mushrooms - growth curves coincide with the axis of Figure 2a abscissa.

Table 1. Diameter of grown colonies (mm) from biocide concentration

Test cultures	Biocide concentration in the nutrient medium, %								
	“Dinovis”				“Megadez”				Control
	0,03	0,06	0,1	0,2	0,03	0,06	0,1	0,2	
<i>Aspergillus niger</i>	-	-	-	-	5	2	-	-	61
<i>Aspergillus spp.</i>	11	3	-	-	6	3	-	-	42
<i>Penicillium spp.</i>	-	-	-	-	-	-	-	-	65
<i>Cladosporium spp.</i>	13	-	-	-	-	-	-	-	73
<i>Mucor spp.</i>	12	-	-	-	7	-	-	-	51

**Figure 1.** Test culture growth kinetics in a control environment: 1 – *Mucor spp.*; 2 – *Cladosporium spp.*; 3 – *Penicillium spp.*; 4 – *Aspergillus spp.*; 5 – *Aspergillus niger*.

In the case of other crops, however, the growth diameter is much lower compared to the control environment: 4.2 times for *Mucor spp.*, 5.6 times for *Cladosporium spp.* and 3.8 times for *Aspergillus spp.*

The increased concentration of Dynovis biocide in the tested systems almost completely suppresses the growth and development of fungi in the nutrient media of Figure 2 b. The only exception is *Aspergillus spp.*, whose growth inhibition compared to the control medium is 14 times.

The use of Megadez in small concentrations provides a greater impact than that of Dynovis (Figure 2 c): the diameter of the colonies is reduced by 7.3 times for *Mucor spp.* and by 7 times for *Aspergillus spp.*, which is 1.7 and 1.8 times higher than the growth of crops in the presence of Dynovis for the indicated fungi. At the same time, the introduction of Megadez leads to a slowdown in crop growth by an average of 3-4 days. The increase in the concentration of the additive in the system, as in the case of Dynovis, almost completely suppresses the development of colonies in the nutrient environment, with the exception of fungi of the *Aspergillus* genus (Figure d). The fungicide activity of Megadez in this case is comparable to that of Dinovis.

The use of the Abbott equation to calculate the coefficients of inhibition of linear growth of microscopic mushroom colonies in the presence of these drugs allowed to quantitatively estimate the value of fungicidality of disinfectants under test.

Analyzing the obtained results, it is worth noting that Megadez and Dinovis can be referred to as strong fungicides (Figure 3), which almost completely suppress mushroom growth.

Regardless of the type and concentration of biocides, the lower limit of the inhibiting factor for mushroom growth is 70%, which indicates the high efficiency of the additives.

Increasing the concentration of biocidal agents to 0.06% leads to an increase in their fungicidal activity, which is expressed by an increase in the coefficient of inhibition of fungi growth, the lower limit of which in this case is 90%. Absolute inhibitory effect of test-cultures is observed at high concentrations.

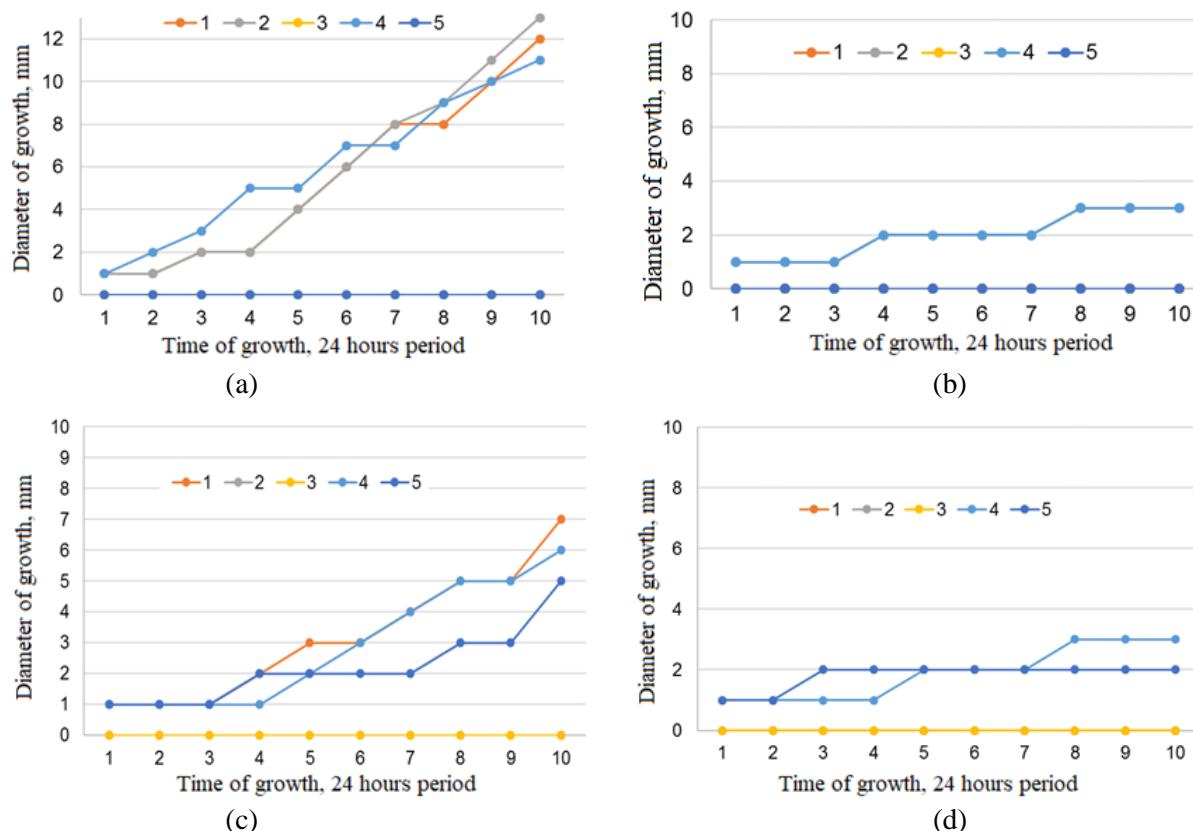


Figure 2. Kinetics of growth of test-cultures in the presence of disinfectants depending on the concentration, where: 1 – *Mucor* spp.; 2 – *Cladosporium* spp.; 3 – *Penicillium* spp.; 4 – *Aspergillus* spp.; 5 – *Aspergillus niger*; (a) Dinovis 0.03 %; (b) Dinovis 0.06 %; (c) Megadez 0.03 %; (d) Megadez 0.06 %.

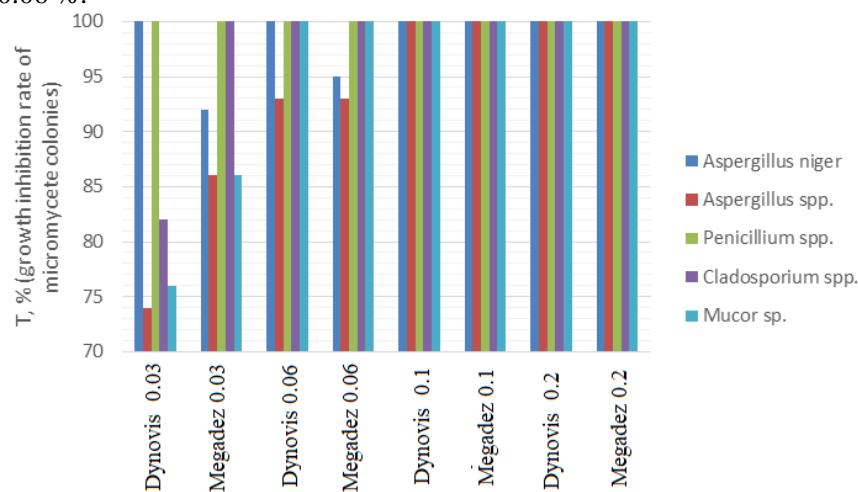


Figure 3. Braking factor of radial growth of micromyctete colonies depending on the type of biocide and its concentration.

Both drugs have a fungicidal effect on all used fungal cultures at concentrations of 0.1% and 0.2%, but when the concentration drops to 0.06%, absolute inhibition does not apply to all crops. Aspergillus spp. slows down growth in the presence of both drugs in the concentration of 0.06%, Aspergillus niger at the same concentration of "Megadez". At the same time, the percentage of growth deceleration for all crops remains high (from 93 to 95%), slightly decreasing when switching to the lowest concentration of

biologically active substances (0.03%), when the lowest value of growth deceleration coefficient of 74% is recorded in *Cladosporium spp.*

4. Conclusion

Thus, the paper assesses the fungicidality of biocidal components of Megadez and Dinovis. Comparable inhibitory effects of two bioactive agents on test cultures are shown. Of all the test cultures, microscopic fungi of the genus *Aspergillus* are more resistant to fungicides, and *Penicillium spp* is the most vulnerable.

The high fungicidality of biocidal agents is due to the synergistic effect of their components (aldehydes, quaternary ammonium compounds, imidazole derivatives), which have different mechanisms of antimicrobial action, which provides a wider range of antifungal activity. On the basis of experimental data for the further researches it is offered to use additives in concentration 0,2 and 0,4 % from weight of cement that corresponds to experimental dosages 0,03 and 0,06 % in a solution at the analysis of fungicidal activity by a method of direct contact with test-cultures. At such concentrations, the required inhibitory capacity of the components is noted in the absence of their overuse and ensuring a minimum negative impact of organic substances on mineral binders.

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