FUNGICIDAL ACTIVITY OF THE BIOCIDAL DRUG "IODOSAN"

Buchkovska H¹ (ORCID ID 0009-0007-4449-614X), Chechet O. ¹, (ORCID ID 0000-0001-5099-5577), Kovalenko V. ¹ (ORCID 0000-0002-2416-5219), Vishchur O. ² (ORCID 0000-0003-4503-3896), Baranov V.¹ (ORCID ID 0009-0004-2372-1326), Zakharin S.³ (ORCID 0000-0002-1263-8170), Asanova M.¹ (ORCID ID 0009-0006-8905-5025)

¹State Scientific and Research Institute for Laboratory Diagnostics and Veterinary and Sanitary Expertize, Kyiv, Ukraine.
²Institute of animal biology NAAS, Lviv, Ukraine.
³State Service of Ukraine on Food Safety and Consumer Protection, Kyiv, Ukraine.
e-mail: galina.buchkovska11@gmail.com

Abstract. The development of modern effective biocidal remedy for poultry, non-toxic disinfection in the presence of poultry with prolonged effect and sanitizing the air environment is a constant task for scientists. Controlling of test cultures of micromycetes is the main indicator of determining the effectiveness of the drug. Various methods are used for this. The results of research on the effect of the biocidal drug "iodosan" based on lactic acid, iodine, isopropanol demonstrated its fungicidal activity against the reference strains of Candida albicans ATCC 10231 and Aspergillus niger ATCC 16404. It is established that by the method of serial dilutions, using paper discs on the Chapek medium and on agar malt extract under the action of the biocidal drug "iodosan" was determined to have an effective concentration of 0.75%.

The biocidal drug «iodosan» in 1.0% concentration after exposure for 60 minutes of effectively disinfected test objects of wood, iron, brick and plaster that were contaminated with micromycetes. It has been proven that the activity of the biocidal drug «iodosan» depends on the concentration, the duration of exposure, which is why it is important to follow to these regimes when using it.

Keywords: biocide, micromycetes, test objects, test microorganisms, fungicidal activity

Bacterial and antifungal safety in the modern poultry industry is one of the main factors of production efficiency. Of course, violation of the requirements of infectious safety and veterinary-sanitary conditions of keeping birds creates not only a serious threat to the productivity of birds, but also the threat of a significant reduction in the number of herds (Boyko, 2016, Kornienko, 2013, Chechet, 2022).

However, in recent years, the difficult economic situation has forced some poultry farms to constantly exploit the same premises for a long time and to introduce sanitary-hygienic measures in a limited way. This leads to an increase in contamination of premises with conditionally pathogenic and pathogenic microflora, the composition and variety of which regularly changes. It was established that high microbial contamination of indoor air can contribute to the occurrence of infection.

Increasing microbial contamination of aviaries promotes the infection of the bird's body, pollution of poultry products, which reduces its quality and can cause human disease. Therefore, the research on microbial contamination, and especially the effect of micromycetes on the bird's body, the development of methods to reduce the microbial background in aviaries are actual tasks in improving production efficiency and the quality of poultry products.

A large number of drugs is offered to combat against microbial contamination of aviaries. However, they have advantages along with their disadvantages, which limits their widespread
usage. The constant increasing production volumes of poultry products and the necessity to improve methods and means of disinfection, especially for use in the presence of birds, leads to research for new effective biocidal agents and the optimization of their application regime.

Disinfection is a part of the technological process in industrial poultry farming. Disinfection of indoor air and equipment surfaces are important factors to combat birds’ diseases (Kornienko, 2013, Wang, 2019).

Active substances such as iodine and lactic acid have a powerful bactericidal, virucidal, fungicidal action. It is known that iodine is a strong oxidizing agent. Due to this, antimicrobial activity is manifested (Kovalenko, 2017, Gebel, 2013). Mechanism of action for organic acids and surfactants, based on changing the surface tension of water and disruption of the cell membrane. The rate of bacterial death is determined by the rate of penetration of disinfectants into the cell and the rate of cell death itself as a result of metabolic disorders (Bondarchuk, 2013, DSTU EN 1275:2004).

Iodine and lactic acid have low toxicity, also they are practically harmless, even at doses 8 times higher than recommended (Bondarchuk, 2013, Kovalenko, 2017, Chechet, 2023, Ibatullin, 2016, Wang, 2019).

Thus, taking into consideration the informational data of the literature and the results of previous researches, it was possible to demonstrate the urgency to control the biocidal agent against fungi-micromycetes and determine its effectiveness (Chechet, 2023, Chechet, 2021).

The main purpose of the research is to determine fungicidal efficiency of the drug «Iodosan» against reference strains of Candida albicans ATCC 10231 and Aspergillus niger ATCC 16404.

Materials and Methodology.

The development and research of the biocidal drug were carried out in the department of State Scientific and Research Institute for Laboratory Diagnostics and Veterinary and Sanitary Expertize. The object of research was the biocidal agent «Iodosan». To conduct research to determine the fungicidal properties of the means, work was done to prepare laboratory glassware, prepare reagents, culture medium and inspection of equipment - thermostats, water baths, microscopes, etc.

The research of the antifungal effect of the drug «Iodosan» and exposure was performed according to generally accepted recommendations: «Metody kontroliu efektyvnosti dii dezinfektantiv na mikromitsety» approved by the Scientific and Methodological Council of the State Committee for Veterinary Medicine (Protocol № 1, 23.12.2009) and the standard DSTU EN 1275:2004 chemical disinfectants and antiseptic basic fungicidal activity. Test method and requirements (stage 1) (Kovalenko, 2017, DSTU EN 1275:2004, Kovalenko, 2016, Kovalenko, 2017, Rutala, 2019). Methods of studying and establishing effective concentrations of the biocidal drug «Iodosan»: suspension and paper discs. Solutions of «Iodosan» agent were prepared in concentrations of 0.3; 0.5; 0.75 and 1.0%. Suspensions of spores were prepared from reference strains of Candida albicans ATCC 10231 (2.5 × 107/cm3) and Aspergillus niger ATCC 16404 (1.8 × 107 cm3). Control of the microorganisms’ culture was investigated in the working dilution. Neutralizing fluid is the environment, used to neutralize the activity of antimicrobial agents in accordance with EF (Himedia, Lot 0000342787; suitable for: May 2022). Neutralizing substances: tvin 80 – 30 g/dm3, licetin 3 g/dm3, histidine hydrochloride 1g/dm3.

Suspension method: 0.1 ml of drug’s solution «Iodosan» was mixed with working dilutions of microorganisms, the contact exposure was 30 and 60 minutes, and seeded on Chapek medium and on agar malt extract. They were placed at a temperature of 27 °C in a thermostat for 14 days. Control of the experiment on days 3, 5, 7, 10 and 14, where the presence or absence of fungal growth was determined (DSTU EN 1275:2004, Kovalenko, 2016, Kovalenko, 2017, Rutala, 2019).

Paper disc method: 0.2 ml of reference strains of Candida albicans ATCC 10231 and Aspergillus niger ATCC 16404 were seeded on Chapek medium and on agar malt extract in petrie dishes. Sterile discs of filter paper (diameter 5 mm) were wetted with aqueous biocide solutions at appropriate concentrations in an amount of 0.1 ml per disc, and laid out in sterile
tweezers on petrie dishes, pressing to the agar. 5 discs were laid out in each petrie dish, which were incubated at a temperature of 27 °C in a thermostat for 10 days. Accounting of results were recorded after 7 and 10 days, also the diameter of latent fungal growth zones around the paper discs was determined using a ruler (DSTU EN 1275:2004, Kovalenko, 2016, Rutala, 2019).

A test objects of iron, plaster, wood and brick was conducted to confirm the final effective concentration of the biocidal drug «Iodosan».

Results.
The following results were obtained by the suspension method during experiments to determine the fungicidal properties of «Iodosan» on test cultures of reference strains of Candida albicans ATCC 10231 and Aspergillus niger ATCC 16404(Table 1).

<table>
<thead>
<tr>
<th>Experimental microorganisms</th>
<th>Control</th>
<th>Concentration of the drug, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0,3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*Note: «+» – presence of fungus growth; «–» – absence of fungus growth.

Solutions of the drug «Iodosan» with a 0.5% concentration at an exposure of 120 minutes demonstrate fungicidal activity, therefore the growth of microorganisms was not observed. It was established that a 0.75% concentration of the drug solution effectively impact on microorganisms at an exposure of 60 minutes.

In the suspension method, the exposure is effective for 60 minutes, therefore, it was used for the research in the method with paper discs (Tables 2, 3). Table 2 demonstrates that on the seventh day, starting from 0.75% concentration, the drug «Iodosan» actively detained the growth of vegetative cells Candida albicans, where the stop growth zone was determined to be more than 9 mm. At 0.75% concentration of the drug, the growth arrest of mold spores’ suspension Aspergillus niger was 7.0 mm.

It was found that «Iodosan» (Table 3) manifested a fungicidal effect in 0.75% concentration on the tenth day, where the growth retardation of Aspergillus niger was 8 mm. Analysing the results, we observe a growth retardation zone up to 12 mm at 1.0% concentration of drug’s. The results of the research demonstrated that with an increase in the concentration of the drug «Iodosan», the growth retardation zone of the fungi Aspergillus niger and Candida albicans increases.

According to DSTU EN 1275:2004 (Table 4), the product passes the standards if the level of bacterial viability decrease is at least 10⁴ during the testing time of not more than 60 minutes at the 20°C temperature under the conditions determined for analysis using test microorganisms Candida albicans ATCC 10231 and Aspergillus niger ATCC 16404. According to this indicator, 0.75% of the drug «Iodosan» provides a fungicidal effect against the reference strains of Candida albicans ATCC 10231 and Aspergillus niger ATCC 16404.
**Table 2**

Fungicidal effect of the drug «Iodosan» using paper discs at an exposure of 60 minutes, (7 days), (M±m, n=5)

<table>
<thead>
<tr>
<th>Experimental microorganisms</th>
<th>Active concentration, %</th>
<th>Diameter of growth retardation zones (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0,3</td>
<td>0,5</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>2,0±0,2</td>
<td>5,0±0,2</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>4,0±0,3</td>
<td>6,0±0,5</td>
</tr>
</tbody>
</table>

**Table 3**

Fungicidal effect of the drug «Iodosan» using paper discs at an exposure of 60 minutes, (10 days), (M±m, n=5)

<table>
<thead>
<tr>
<th>Experimental microorganisms</th>
<th>Active concentration, %</th>
<th>Diameter of growth retardation zones (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0,3</td>
<td>0,5</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>4,0±0,2</td>
<td>6,0±0,2</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>6,0±0,3</td>
<td>9,0±0,5</td>
</tr>
</tbody>
</table>

100% disinfection of Candida albicans ATCC 10231 and Aspergillus niger ATCC 16404 were achieved by the biocidal drug «Iodosan» in 0.75% concentration and exposure for 60 min on test objects made of wood, iron, brick and plaster (Table 5). It confirms the effectiveness of this concentration for usage in production.

**Table 4**

Fungicidal activity of the drug «Iodosan»

<table>
<thead>
<tr>
<th>Strains of microorganisms</th>
<th>Concentration, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0,75</td>
</tr>
<tr>
<td>Candida albicans ATCC 10231</td>
<td>2,5×10^7</td>
</tr>
<tr>
<td>Aspergillus niger ATCC 16404</td>
<td>1,8×10^7</td>
</tr>
</tbody>
</table>

Indicator of a decrease in the level of viability of vegetative cells Candida albicans and mold spore suspension Aspergillus niger at the specified test product concentrations.

<table>
<thead>
<tr>
<th>Concentration, %</th>
<th>0,75</th>
<th>1,0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 minutes</td>
<td>30 minutes</td>
</tr>
<tr>
<td>Candida albicans ATCC 10231</td>
<td>1,7×10^7</td>
<td>4,8×10^6</td>
</tr>
<tr>
<td>Aspergillus niger ATCC 16404</td>
<td>1,5×10^7</td>
<td>6,0×10^6</td>
</tr>
</tbody>
</table>

Disinfection of livestock premises in infectious diseases caused by fungal microorganisms by wiping, irrigation in relation to the reference strains of Candida albicans ATCC 10231 and Aspergillus niger ATCC 16404. It is recommended to use a working solution of the drug «Iodosan» with a 0.75% concentration at the rate of 0.3-0.4 liters per 1 m² at an exposure of 60 min.
The mechanism of fungicidal effect of the drug can explain the synergistic effect on the microbial cell observed with disinfecting the surface of a complex of chemical, active substances of the drug (Wang, 2019, Kovalenko, 2013, Rutala, 2019). The results of the research established an assessment of the effectiveness of the control over method micromycetes, which is confirmed by two methods (Tomasino, 2013, Kovalenko, 2013, Chechet, 2022). The difference in methods is associated with more strict and modern requirements for research and evaluation of results. The obtained results meet the requirements of qualitative and quantitative determination of the experimental information and accompanying controls, which is more modern and guarantees their reproducibility (Podzerey, 2008, Rutala, 2019).

Table 5

<table>
<thead>
<tr>
<th>Fungicidal effect of the drug «Iodosan» (1.0%) at an exposure of 60 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microbiological culture</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>100</td>
</tr>
</tbody>
</table>

Thus, the effectiveness of the fight against infectious diseases in poultry industry and their prevention depend on the quality of the conducted disinfection. The quality of biocidal drugs on a pathogenic microorganism depends on factors such as: fungicidal properties of the biocide, concentration, temperature of solutions, premises’ temperature, the amount of solution that is consumed to disinfect the object, exposure on the infecting agent and, of course, the biological properties of the microorganism (Kovalenko, 2017, Tomasino, 2013, Zwirzz, 2020, Ortiz, 2015, Wang, 2019).

The next stage of the research of the biocidal drug “Iodosan” in the conditions of production in the presence of birds.

**Conclusions.** Researches have established that the biocidal drug “Iodosan” at a concentration of 0.75% has a fungicidal effect on the reference strains of Candida albicans ATCC 10231 and Aspergillus niger ATCC 16404.

A solution of the biocidal drug “Iodosan” in 1.0% concentration at an exposure of 60 minutes demonstrates fungicidal properties on test objects.

**References**


ФУНГІЦІДНА АКТИВНІСТЬ БІОЦИДНОГО ПРЕПАРАТУ «ЙОДОСАН»

Бучковська Г. А.1 (ORCID ID 0009-0007-4449-614X), Чечет О. М.1, (ORCID ID 0000-0001-5099-5577), Коваленко В. Л.1 (ORCID ID 0000-0002-2416-5219), Віщур О. І.2 (ORCID ID 0000-0003-4503-3896), Баранов Б. С.1 (ORCID ID 0009-0004-2372-1326), Захарін С. В.3 (ORCID ID 0000-0002-1263-8170), Асанова М. Р.1 (ORCID ID 0009-0006-8905-5025)

1Державний науково-дослідний інститут з лабораторної діагностики та ветеринарно-санітарної експертизи, м. Київ, Україна;
2Інститут біології тварин НААН, м. Львів, Україна;
3Державна служба України з питань безпечності харчових продуктів та захисту споживачів, м. Київ, Україна.

e-mail: galina.buchkovska11@gmail.com

Резюме. Розробка сучасних ефективних біоцидних засобів для птахівництва, нетоксичних за дезінфекції у присутності птиці із пролонгованою дією та сануючи повітряне середовище постійна задача для науковців. Контролювання на тест-культурах мікроміцетів – це головний показник визначення ефективності препарату. Для цього застосовують різні методи. Результати досліджень впливу біоцидного препарату «Йодосан» на основі молочної кислоти, йоду, ізопропанолу показали його фунгіцидну активність щодо еталонних штамів Candida albicans ATCC 10231 і Aspergillus niger ATCC 16404. Встановлено, що методом серійних розведення, з використанням паперових дисків на середовищі Чапека та на агарі солодового екстракту за дії біоцидного препарату «Йодосан» визначена ефективною 0,75 % концентрація. Біоцидний препарат «Йодосан» у 1,00 % концентрації за експозиції 60 хв ефективно знизав рост і плодоношення мікроміцетів, що були контаміновані тест-об’єктами. Доведено, що активність біоцидного препарatu «Йодосан» залежить від концентрації, терміну експозиції, що важливо дотримуватися даних режимів при застосуванні.

Ключові слова: біоцид, мікроміцети, тест-об’єкти, тест-мікроорганізми, фунгіцидна активність

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