DETERMINATION OF ANTIMICROBIAL AND FUNGICIDAL PROPERTIES OF EXPERIMENTAL DISINFECTANT «SUKHODEZ»

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Abstract: The results of the study of antimicrobial and fungicidal properties of the experimental preparation "Sukhodez" against microorganisms *E. coli*, *Salmonella enteritidis*, *Staphylococcus aureus* and fungi *Candida albicans* in the laboratory are presented.

The aim of the research. To study the antimicrobial and fungicidal properties of powdered disinfectant and analyze the prospects for its further use in a set of anti-epizootic measures in particular in poultry farms.

Materials and methods. The research was conducted during 2021 at the Department of Veterinary Examination, Microbiology, Zoohygiene and Safety and Quality of Animal Products of the Faculty of Veterinary Medicine of Sumy National Agrarian University. Evaluation of bactericidal properties of the experimental agent "Sukhodez" was determined on museum strains of E. coli ATCC 25922, Salmonella enteritidis ATCC 13076 and Staphylococcus aureus ATCC 6538, fungicidal properties were studied on fungi Candida albicans. All cultures were standardized to 109 CFU/cm^3.

Results. It is established that as a result of the conducted researches at studying preparation "Sukhodez" antimicrobic properties, it had high efficiency concerning action on strains of both gram-positive and gram-negative bacteria. It had a detrimental effect on bacteria when applied to concrete at an exposure of 1 hour with a rate of 75 mg per 1 m^2, and had the same effect as when applied to a wooden surface, where it expressed antimicrobial action at a rate of 50 mg/m^2 after 4 hours. When determining the fungicidal properties, it was found that when exposed to 5 hours, the preparation "Sukhodez" had an effect on study contaminated objects with a rate of 50 mg/m^2, and with a rate of 75 mg/m^2 inhibited the growth of fungi for 1 hour.

Conclusions. It was found that the most sensitive to the preparation "Sukhodez" were the culture of Salmonella enteritidis – at a rate of 25 g/m^2 and action on concrete and wooden surfaces, the disinfectant inhibited the growth of the culture when exposed to 5 hours, and at a rate of 50 g/m^2 – for 2 years. Staphylococcus aureus was the most stable bacterial culture, so at a rate of 50 g/m^2 on concrete and wood surfaces, bacterial growth was inhibited for 4 and 5 hours, respectively. At the same time, in the study of fungicidal properties, "Sukhodez" showed a fungicidal effect on Candida albicans when applied to a concrete surface at a rate of 50 mg/m^2 for 3 hours, when applied to a wooden surface – for 4 hours.

In general, at a rate of 75 g/m^2, "Sukhodez" has an instant bactericidal and fungicidal effect on bacteria and fungi applied to concrete and wooden surfaces.

Keywords: disinfection, antimicrobial and fungicidal activity, disinfectant, laboratory, microorganisms, poultry, "Sukhodez".

1. Introduction

Poultry farming is one of the most promising and fastest growing branches of business, and poultry is one of the world's largest stocks of domestic animals [1, 2]. Thus, ensuring the biosecurity of birds is mandatory for economic prosperity and public health [3, 4]. The main purpose of disinfection is to break the epizootic chain by influencing the transmission factor of the pathogen from the source of infection to the susceptible organism. The experimental preparation "Sukhodez" was developed for use as a dry disinfectant for livestock facilities, and in particular on poultry farms.

Preventive measures, including cleaning and disinfection, are fundamental steps for biosecurity programs and indispensable for maintaining high productivity on poultry and livestock facilities [5]. Proper use of universal disinfectants is a major part of the program to protect animals and humans [6].

Recent reports have shown that most poultry farms do not practice the basic principles of biosecurity [7]. Spraying disinfectants in barns and removing feces were the only sanitation schemes adopted in farms [8, 9]. These disinfectants are used without regular inspection and evaluation of effectiveness, while the effectiveness of disinfectants is influenced by the composition, level of organic matter, humidity, temperature, dilution rate, pH and hardness of water and other factors [10]. In addition, the use of disinfectants without validation and evaluation can lead to high selective pressure, which will gradually reduce the sensitivity of organisms to the disinfectants used and even cross-resistance to antibiotics of health concern. Thus, inadequate sanitation procedures can be ineffective in controlling diseases, which reduces the productivity of birds [11]. Therefore, the evaluation of the effectiveness of disinfectants should be a priority for the selection of the appropriate disinfectant by minimizing the microbial.

When choosing a disinfectant for livestock, poultry farmers should pay attention to a number of characteristics that must have a disinfectant, and in particular its effect on the condition, health, behaviour of animals and birds [12]. Numerous engineering standards are strictly adhered to limit the spread of pathogens in animal housing [13]. Chemical disinfectants are often the first line of defense against these pathogens and are a must when choosing the right product.

Thus, disinfection of livestock and poultry facilities is one of the highest priority on the path to a healthy and prosperous economy. Widely used chemical disinfectants for broiler premises include available chlorine, ozone, Quaternary ammonium salt and glutaraldehyde. Different disinfectants used for large-scale disinfection of broiler houses work through different mechanisms, and therefore, the effectiveness of their disinfection also varies. Chlorine-containing compounds (sodium dichloroisocyanurate, sodium hypochlorite, bleaching powder, chlorine dioxide, etc.) are widely used as disinfectants in livestock and poultry [14].

Due to the fact that a significant number of disinfectants used are toxic, exhibit immunosuppressive properties for animals and poultry, it is still relevant for modern veterinary medicine to develop new, safe and effective means of disinfection [15, 16].

Given the above, the development of disinfectants based on nanotechnology, the components of which have a wide range of action (antibacterial, antiviral, antifungal action), high biological activity and low toxicity, is an indisputable alternative to traditional disinfectants [17].

Many disinfectants are used on farms by aerosol splicing, which is difficult to do in the presence of animals and poultry. Also using aerosol products, in addition to heavy and costly use, the premises accumulate excess moisture. Therefore, we offer current disinfection of premises with dry biocidal products. These disinfectants by their properties are environmentally friendly, have a loose composi-

tion, the vast majority of light color, pleasant aroma, and most importantly – most have an adsorbent effect. They are effective for the destruction and control of many bacteria, viruses, fungi, parasites, larvae of flies. In addition, they improve the quality of litter, reduce ammonia content and humidity in livestock premises [18]. Thus, the development of new highly effective, cheap, multifunctional, as well as environmentally friendly disinfectants, is an important and priority area of research in the field of veterinary sanitation [19].

Powdered disinfectants are convenient to use not only when they can be used in the presence of animals and birds, but also due to their partially adsorbing action [20]. As a result: the property of the drug to absorb moisture from the litter prevents many diseases associated with excessively wet litter (dermatitis, coccidiosis and some infections). It is especially important that reducing the release of ammonia, hydrogen sulfide and other harmful gases into the atmosphere of livestock and poultry significantly improves the health of animals and poultry, reduces treatment costs, more complete and efficient use of feed, improves working conditions for staff. All this gives grounds to reduce the environmental tax on agricultural enterprises as a result of the fact that emissions of ammonia and other pollutants are significantly reduced [21].

The aim of the research. To study antimicrobial and fungicidal properties of experimental powdered disinfectant and to analyze prospects of its further application in a complex of antiepizootic measures in particular on poultry farms.

2. Materials and methods

Evaluation of bactericidal properties of the experimental agent "Sukhodez" was determined on museum strains of *E. coli* ATCC 25922, *Salmonella enteritidis* ATCC 13076 and *Staphylococcus aureus* ATCC 6538, fungicidal properties were studied on fungi *Candida albicans*.

The composition of the active substance of the experimental disinfectant "Sukhodez" (%): chloramine – 0.2; thymol – 0.1; copper sulfate – 2.0; iron sulfate – 1.0; calcium sulfate dihydrate – 45.0; zeolite – 42.0; kaolin – 9.6; flavoring – 0.1.

All cultures were standardized to 10^9 CFU/cm^3 [22]. The concentration of bacterial cells in 1 cm^3 of the working suspension was determined using the bacterial turbidity standard. After the tested exposures, the effect of the chemicals was neutralized with sodium hydroxide. Suspension of each culture was sprayed on concrete and wooden surfaces of test objects at the rate of 5 cm^3/100 cm^2.

An ovalbumin solution was used to determine the effect of protein load on the level of antimicrobial activity. To do this, after drying the suspension, the test objects were treated with a solution of ovalbumin – 20 mg/cm^3 (protein protection) and applied the preparation "Sukhodez" at the rates of consumption: 25, 50, 75 and 100 g/m^2. As a control, the infected test object was treated with boiled tap water. After 1, 2, 3, 4, 5 and 6 hours of exposure, washes from test objects were performed on an area of 10×10 cm (100 cm^2). For which samples were taken with sterile cotton swabs, chemicals were neutralized and additionally centrifuged twice for 30 min at 2500 rpm. The precipitate after the second centrifugation was diluted with 1 cm^3 of sterile saline and sown in 0.5 cm3 on KODA medium (objects contaminated with *E. coli*) and BCH and Saburo agar (objects contaminated with *C. albicans*). The cultures were incubated at 37 °C for 2 days. Accounting for the bactericidal and fungicidal action of the experimental agent was carried out by changing the color of the medium and the presence or absence of growth on nutrient media in the experiment and control. The test was considered positive when the color of the KODA medium changed from green to yellow, when the BCH was cloudy and there was growth on Saburo medium.

3. Results

When studying the effectiveness of the experimental disinfectant "Sukhodez" on the pathogens of the most common infectious and fungal diseases that were on the test objects (concrete, wood) in direct contact (**Tables 1, 2**), it was found that the experimental preparation "Sukhodez" was effective against a controlled spectrum of pathogens of bacterial diseases and had an adsorbing effect.

Table 1

The effectiveness of the experimental preparation "Sukhodez" on the cells of *E. coli* bacteria ATCC 25922, *Salmonella enteritidis* ATCC 13076 and *Staphylococcus aureus* ATCC 6538, which were applied to concrete

Exposition	Test cultures and norms of consumption of preparation, g/m^2											
	Salmonella enteritidis ATCC 13076				*Staphylococcus aureus* ATCC 6538				*Escherichia coli* ATCC 25922			
	25	50	75	100	25	50	75	100	25	50	75	100
60 min	✓	✓	×	×	✓	✓	×	×	✓	✓	×	×
120 min	✓	✓	×	×	✓	✓	×	×	✓	✓	×	×
180 min	✓	×	×	×	✓	✓	×	×	✓	✓	×	×
240 min	✓	×	×	×	✓	×	×	×	✓	×	×	×
300 min	×	×	×	×	✓	×	×	×	✓	×	×	×
360 min	×	×	×	×	✓	×	×	×	×	×	×	×

Notes: «✓» – growth of cultures; «×» – absence of culture growth.

At a consumption rate of 75 g/m^2, uniform sprinkling of test objects contaminated with suspensions of cultures: *E. coli* ATCC 25922, *Salmonella enteritidis* ATCC 13076 and *Staphylococcus aureus* ATCC 6538, within the first hour after application provided 100 % disinfection. A smaller dose of this tool (50 g/m^2) also provided the same efficiency for 3–4 hours.

Table 2

The effectiveness of the experimental preparation "Sukhodez" on the cells of *E. coli* bacteria ATCC 25922, *Salmonella enteritidis* ATCC 13076 and *Staphylococcus aureus* ATCC 6538, which were applied to the wood

Exposition	Test cultures and norms of consumption of preparation, g/m^2											
	Salmonella enteritidis ATCC 13076				*Staphylococcus aureus* ATCC 6538				*Escherichia coli* ATCC 25922			
	25	50	75	100	25	50	75	100	25	50	75	100
60 min	✓	✓	×	×	✓	✓	×	×	✓	✓	×	×
120 min	✓	✓	×	×	✓	✓	×	×	✓	✓	×	×
180 min	✓	✓	×	×	✓	✓	×	×	✓	✓	×	×
240 min	✓	×	×	×	✓	✓	×	×	✓	✓	×	×
300 min	×	×	×	×	✓	×	×	×	✓	×	×	×
360 min	×	×	×	×	✓	×	×	×	×	×	×	×

Notes: «✓» – growth of cultures; «×» – absence of culture growth.

When determining the fungicidal properties, it was proved that the experimental preparation "Sukhodez" has a pro-

nounced fungicidal effect at a rate of 75 g/m^2 and an exposure of 1 hour and 50 g/m^2 at an exposure of 4 hours (**Table 3**).

Table 3

The effectiveness of the experimental preparation "Sukhodez" on the cells of the fungi *Candida albicans*, which were applied to test objects

Exposition	Test objects							
	wood				concrete			
	norms of consumption of preparation, g/m^2							
	25	50	75	100	25	50	75	100
60 min	✓	✓	×	×	✓	✓	×	×
120 min	✓	✓	×	×	✓	✓	×	×
180 min	✓	✓	×	×	✓	✓	×	×
240 min	✓	✓	×	×	✓	×	×	×
300 min	×	×	×	×	✓	×	×	×
360 min	×	×	×	×	×	×	×	×

Notes: «✓» – growth of cultures; «×» – absence of culture growth.

4. Discussion of research results

Dry disinfection plays an important role in the system of veterinary and sanitary measures on farms, because in contrast to aerosol and wet disinfection, dry disinfectants could be used in the presence of animals and poultry [23].

In this regard, a new experimental preparation "Sukhodez" was developed.

The antibacterial and fungicidal properties of "Sukhodez" when exposed to test surfaces contaminated with culture suspensions were studied: *E. coli* ATCC 25922, *Salmonella enteritidis* ATCC 13076 and Staphylococcus aureus ATCC 6538, fungi *Candida albicans*. However, it was found that when used on concrete and wooden surfaces at a rate of 75 g/m^2, the preparation has a detrimental effect on microorganisms *E. coli*, *Salmonella enteritidis*, Staphylococcus aureus and fungi *Candida albicans* [24].

On the basis of the carried-out laboratory researches the optimum indicators of exposure and expenses of the working means "Sukhodez", from calculation of weight on m^2 of the area of the room which is subject to disinfection are developed and established.

Summarizing the obtained scientific results, it should be noted that our research was aimed at studying the antimicrobial and fungicidal properties of the experimental preparation "Sukhodez" which should be used in livestock facilities in the presence of animals.

Study limitations. The limitation of the research is to conduct experiments in the laboratory on museum strains that are different from the field, so the concentration of the drug may vary slightly, depending on the level of contamination.

Prospects for further research. Further work will be aimed at study and testing the results of virucidal properties of dry disinfectant "Sukhodez".

5. Conclusions

Thus it is proved that the experimental drug "Sukhodez" at a consumption rate of 75 g/m^2 has a pronounced bactericidal and fungicidal action against grams of negative, grams of positive microorganisms and fungi at an exposure of 1 hour, a similar effect has a consumption rate of 50 g/m^2 at exposure for 3–4 hours when applying test cultures on both concrete and wood.

Conflict of interests

The authors declare there is no conflict of interests.

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