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DETERMINATION OF FUNGICIDAL EFFECT OF DISINFECTANT 'SANDEZVET' ON SANITARY SIGNIFICANT TEST CULTURES OF MOLD MICROMYCETES OF THE GENUS *ASPERGILLUS* MICH.

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Summary. Studying the properties of new disinfectants is a crucial aspect of disinfectology research. During the investigation of the fungicidal properties of the 'SanDezVet' disinfectant, it was discovered that the recommended concentrations of 0.1% and 0.5%, at a temperature of 20.0 ± 0.5 °C and an exposure time of 60, 120, and 180 minutes, do not inhibit the growth of *Aspergillus fumigatus*, *Aspergillus flavus*, and *Aspergillus niger* test cultures. This conclusion was reached as continuous growth of micromycetes was observed in all drug dilutions. The disinfectant 'SanDezVet' at a concentration of 3.0% displayed fungistatic properties by significantly delaying the growth of experimental test cultures. A 5.0% solution of the disinfectant resulted in a complete delay in growth of museum strains of *Aspergillus fumigatus*, *Aspergillus flavus*, and *Aspergillus niger*. This indicates fungicidal properties when compared to the positive control. Based on the obtained results, it was observed that the optimal exposure times for disinfection measures with 'SanDezVet' are 60 and 120 minutes. 'SanDezVet' can be effectively used for fungal infections at different veterinary facilities

Keywords: *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*

Introduction. Aspergillosis, an acute and chronic mycosis, is an infectious disease affecting birds, animals, and humans. It is characterized by the formation of fibrinous nodular lesions in the respiratory system and serous membranes. This disease is prevalent worldwide and is known to cause significant economic losses to poultry farms due to high poultry mortality rates ranging from 40–90% (Kuznetsov, 2018; Cadena, Thompson and Patterson, 2016). The primary causative agent of aspergillosis in poultry and mammals is the fungus *Aspergillius fumigatus*. In certain instances, *A. flavus, A. niger*, and *A. nidulans* are responsible for significant outbreaks of aspergillosis in chickens and turkeys. *Aspergillus* fungi are prevalent in the environment as saprophytes and become parasitic when ingested under favorable conditions, thereby acquiring pathogenic properties (Chander, 2018; Sharma et al., 2013).

Favorable sanitary and hygienic conditions in poultry housing and feeding are of primary importance in preventing aspergillosis. The use of veterinary and sanitary disinfection measures is essential in increasing productivity and maintaining sanitary quality of products, raw materials, and animal feed (Kuznetsov, 2018; Cadena, Thompson and Patterson, 2016).

Scientific evidence and practical experience suggest that disinfection measures, including disinfection, disinsection, deratization, and sterilization, have proven to be the most cost-effective, affordable, and reliable means of prevention (Vershniak, 2010).

In the context of socio-economic transformations, the role of disinfection in ensuring human safety and health has significantly increased. Disinfection is a vital component in the comprehensive approach to combating contagious diseases. Due to various reasons, disinfection measures are becoming progressively crucial in preventing and eliminating infections. Inadequate funding and the resulting challenges of maintaining sanitary and antiepidemic protocols are the most significant of these circumstances.

It should also be noted that the microbial background has changed as a result of adaptation to the drugs used. Strains of microorganisms that are resistant to traditional disinfectants are increasingly being identified. Environmental safety issues have also been raised recently. The increase in disinfection should not be accompanied by an increase in the release of hazardous chemicals into the environment. For the optimal solution of the above problems related to the need for disinfection in veterinary practice, modern, highly effective disinfectants and antiseptics are needed. Without modern disinfectants, it is impossible to ensure the necessary sanitary and epidemiological regime and reliable protection against infections in farms (Vershniak, 2010; Bordunova et al., 2021).

In view of the above, the study of the properties of new disinfectants is an important area of disinfectology. Therefore, our goal was to determine the fungicidal properties of the new disinfectant 'SanDezVet' on sanitary significant cultures of molds of the genus *Aspergillus* Mich. The study was conducted in the Laboratory of Toxicological Monitoring, Clinical Biochemistry, Safety and Quality of Agricultural Products of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine'.

Materials and methods. The object of the research was the disinfectant 'SefDezInstru', which was first used in veterinary practice in poultry farming under the name 'SanDezVet'.

The research was conducted using commonly accepted procedures for mycological analysis and guidelines for establishing fungicidal properties and optimal disinfectant regimens with *Aspergillus* Mich. test cultures (Seliber, 1962; Yaroshenko et al., 2009; Semenov, 1990). The species affiliation was determined through a comparison of cultural and morphological characteristics, such as the external features of microbial colonies, growth features, color, shape, consistency of colonies, and the presence or absence of sclerotia and pigment. This was done by referencing the descriptions provided in the microbial identifiers and utilizing museum strains of test cultures (Bilay and Koval', 1988; Pidoplichko, 1972).

Mathematical processing of the results was performed using the methods of variation statistics. To calculate the results of the inoculation, all colonies of test cultures of micromycetes that grew in Petri dishes were counted.

The dishes with continuous growth of test culture spores on more than half of the agar surface area, uneven distribution of colonies on the nutrient medium, and those that could not be counted were not taken into account.

The average number of fungal colonies was determined by repeating the experiment three times using the effective parameters of the disinfectant (concentration, temperature, and exposure) and counting the colonies each time. The results obtained were used to calculate the average number of colonies, draw a series of variations, and determine the median. The highest dilution of the drug was effective, as evidenced by at least three experiments that resulted in the death of 95–98% of the spores in the test culture, while the control spores continued to grow. After testing several concentrations, the minimum concentration with the greatest effect on growth retardation of the test culture under optimal temperature and exposure conditions was recommended for use (Semenov, 1990).

Results and discussion. The fungicidal properties of 'SanDezVet' disinfectant were assessed at the recommended concentrations of 0.1, 0.5, 1.0, 3.0, and 5.0% against the most resilient and hygienically significant test cultures of the *Aspergillus* Mich. genus, including *Aspergillus fumigatus*, *Aspergillus flavus*, and *Aspergillus niger,* standardized by spore count. The exposure duration was 60, 120, and 180 min, while the temperature was maintained at 20 ± 0.5 °C.

The fungicidal properties of 'SanDezVet' at various concentrations (0.1, 0.5, 1.0, 3.0, and 5.0%) on *Aspergillus fumigatus* test cultures are shown in Table 1.

Table 1 — Fungicidal activity of 'SanDezVet' against *A. fumigatus* at a temperature of 20 ± 0.5 °C

'SanDezVet' concentrations, %	Terms for calculating the growth of *A. fumigatus* colonies, days														
	3			5			7			10			14		
	Time exposure, min														
	60	120	180	60	120	180	60	120	180	60	120	180	60	120	180
	Number of colonies that grew, pcs.														
0.1	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+
0.5	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+
1.0	–	–	–	+	+	107	+	+	109	+	+	109	+	+	109
3.0	–	–	–	37	17	–	38	18	–	38	19	–	39	19	–
5.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Positive control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Negative control with nystatin	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Notes: '–' — no growth; '+' — continuous growth.

The results obtained (Table 1) indicate that exposure of the test culture to 0.1, 0.5, and 1.0% solutions at 20 ± 0.5 °C for 60, 120, and 180 min did not affect the decrease in the number of *A. fumigatus* colonies — continuous growth of the micromycete was observed (Fig. 1A). A 1.0% concentration of the drug solution at 180 min exposure showed insignificant fungistatic properties. Whereas 3.0% solution of 'SanDezVet' showed significant fungistatic properties on the test cultures of *A. fumigatus*. The 5.0% solution concentration resulted in a complete growth delay in the test cultures and exhibited fungicidal properties relative to the positive control.

Table 2 shows the results of the evaluation of the fungicidal effects of the 'SanDezVet' disinfectant on *A. flavus* test cultures at concentrations of 0.1, 0.5, 1.0, 3.0, and 5.0%. The data presented in Table 2 demonstrate that exposing the test culture to 0.1, 0.5, and 1.0% solutions for 60, 120, and 180 min at 20 ± 0.5 °C did not have any significant effect on decreasing the number of *A. flavus* colonies; instead, we observed a continuous growth in the micromycete as illustrated in Fig. 1B.

The 1.0% concentration affected the number of colonies that grew after exposure for 180 min, i.e., it showed insignificant fungistatic properties. At 3.0% concentration, 'SanDezVet' solution inhibited the growth of the test culture of *A. flavus*, indicating its fungistatic properties. When using a 5.0% solution, the drug 100% inhibited the growth of the test culture and showed fungicidal properties compared to the positive control.

The results of determining the fungicidal properties of the disinfectant 'SanDezVet' at concentrations of 0.1, 0.5, 1.0, 3.0 and 5.0% on the test culture of *A. niger* are presented in Table 3.

The results obtained indicate that exposure of the test culture to 0.1, 0.5, and 1.0% solutions at (20.0 ± 0.5) °C for 60, 120, and 180 min did not affect the growth retardation of *A. niger* colonies — continuous growth of the test culture was found (Fig. 1C).

The 1.0% concentration at 180 min exposure showed insignificant fungistatic properties — a decrease in the number of colonies was observed.

'SanDezVet' in 3.0% concentration influenced a significant delay in the growth of the test culture of *A. niger* — fungistatic properties were manifested, and 5.0% solution contributed to a complete delay in the growth of the test culture, i.e. it showed fungicidal properties compared to the positive control.

Thus, analyzing the results of Tables 1–3, it should be noted that the recommended concentrations of 0.1–1.0%, temperature parameters 20.0 ± 0.5 °C for exposure time of 60, 120, and 180 min did not affect the growth retardation of test cultures of *A. fumigatus, A. flavus, A. niger*, since in all dilutions of the preparation there was a continuous growth of micromycetes, 'SanDezVet' at 3.0% concentration showed fungistatic properties, and 5.0% — fungicidal properties — complete growth retardation of museum strains of test cultures in comparison with the positive control.

Table 2 — Fungicidal activity of 'SanDezVet' against *A. flavus* at a temperature of 20 ± 0.5 °C

'SanDezVet' concentrations, %	Terms for calculating the growth of *A. flavus* colonies, days														
	3			5			7			10			14		
	Time exposure, min														
	60	120	180	60	120	180	60	120	180	60	120	180	60	120	180
	Number of colonies that grew, pcs.														
0.1	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+
0.5	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+
1.0	–	–	–	+	+	91	+	+	95	+	+	99	+	+	99
3.0	–	–	–	28	10	–	29	11	–	30	13	–	31	15	–
5.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Positive control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Negative control with nystatin	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Notes: '–' — no growth; '+' — continuous growth.

Table 3 — Fungicidal activity of 'SanDezVet' against *A. niger* at a temperature of 20 ± 0.5 °C

'SanDezVet' concentrations, %	Terms for calculating the growth of *A. niger* colonies, days														
	3			5			7			10			14		
	Time exposure, min														
	60	120	180	60	120	180	60	120	180	60	120	180	60	120	180
	Number of colonies that grew, pcs.														
0.1	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+
0.5	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+
1.0	–	–	–	+	+	88	+	+	89	+	+	89	+	+	89
3.0	–	–	–	25	14	–	29	16	–	29	18	–	29	19	–
5.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Positive control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Negative control with nystatin	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Notes: '–' — no growth; '+' — continuous growth.

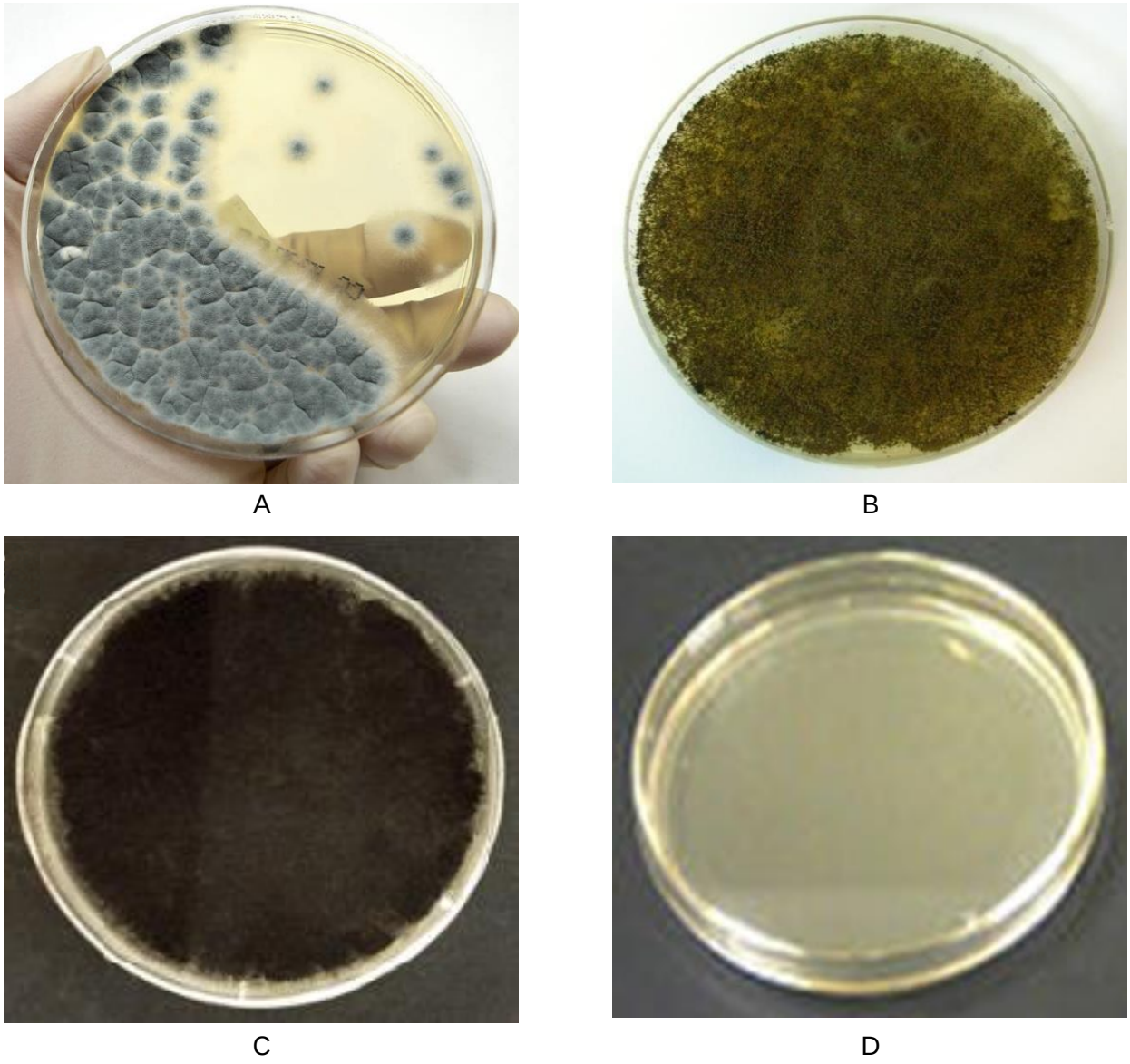

Figure 1. Continuous growth of a 7-day test cultures of *A. fumigatus* (A), *A. flavus* (B), and *A. niger* (C) in 0.5% 'SanDezVet' solution after 180 min exposure compared to the negative control (D).

After analyzing the results, they were statistically processed for the 3.0% solution, the dilution that showed the highest fungistatic properties on all experimental test cultures (Table 4). Comparing the results of the experiments, it should be noted that the most optimal exposure times for 'SanDezVet' disinfection were 60 and 120 min.

Thus, based on the results obtained, 'SanDezVet' can be used for infections of fungal etiology and in different veterinary facilities.

Table 4 — Statistical processing of the results of experiments to determine the fungistatic properties of 3.0% solution 'SanDezVet' against representatives of the genus Aspergillus Mich. at a temperature of 20.0 ± 0.5 °C

Exposure, min	Name of the test culture	Variational range	Average indicator of colonies that have grown	Median
60	*A. fumigatus*	0; 37; 38; 38; 39	30.4	38.0
	A. flavus	0; 28; 29; 30; 31	23.6	29.5
	A. niger	0; 25; 27; 29; 29	22.0	28.0
120	*A. fumigatus*	0; 17; 18: 19; 19	14.6	18.5
	A. flavus	0;10; 11: 13;15	9.8	12.0
	A. niger	0; 14; 16; 18; 19	13.4	17.0

Conclusions. 1. 'SanDezVet' drug in concentrations of 0.1 and 0.5%, at a temperature of 20.0 ± 0.5 °C and exposure for 60, 120, and 180 min had no effect on the test cultures of *Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger* — in all dilutions of the drug, continuous growth of micromycetes was observed.

2. The fungicidal properties of 'SanDezVet' at 3.0% concentration were determined — a significant delay in the growth of experimental test cultures was observed; 5.0% solution of the disinfectant contributed to a complete delay in the growth of museum strains of *Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger* — i.e. it showed 100% fungicidal properties in comparison with the positive control.

Prospects for further research are to study the decontamination concentrations of the disinfectant against museum strains of the test cultures *Penicillium divaricata, Penicillium asymmetrica, Penicillium monoverticillata, Penicillium biverticillata*.

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