

# Disinfection of beekeeping objects with oxygen-containing preparations for bee aspergillosis

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**Abstract.** Aspergillosis of bees is an infectious disease of bee brood and adult bees caused by fungi of the *Aspergillus* genus. These fungi live and are reproduced in soil, feeding on plant material, including the stamens and nectary of flowers. The causative agents are brought into hives by bees carrying nectar and pollen. Once inside, they grow on honeycomb, bee bread, larvae, pupae, and adult bees under favorable conditions. Aspergillosis is a zoonoanthroposis, causing significant economic damage to beekeeping and potentially posing a risk to consumers of bee-derived products. Therefore, important tasks are the selection of preparations that meet the requirements of the industry and the development of effective regimens and applications. This paper presents the results of laboratory and apiary trials of oxygen-containing preparations for the disinfection of bee-keeping facilities (hives, frames, equipment) in cases of aspergillosis. Based on the data obtained, effective regimes of their use are recommended.

## 1 Introduction

Aspergillosis is a fungal disease that affects adult bees and their brood. It is caused by fungi from the *Aspergillus* genus and occurs sporadically, mainly in the spring. It is a toxicomycosis that has both toxigenic and pathogenic properties, which can infect the living bodies of larvae and adult bees. The following species of *Aspergillus* are pathogenic to honey bees: *A. flavus* Link, *A. niger* Tieghem, and *A. fumigatus* Fres. Among these, *A. flavus* is the most common cause of damage to honey bees. *A. fumigatus* and *A. niger* are slightly less common. Infection occurs when larvae and bees consume spore-contaminated honey, nectar, and pollen through their intestines. The percentage of bee deaths varies depending on the conditions and strength of the colony [1-3].

For the first time, bee aspergillosis was discovered in the USA (Texas) by W.K. Howard in 1894. The researcher described the disease of unsealed brood. Subsequently, they detected damage in pupae and adult bees. In Germany aspergillosis was reported by A. Maassen. This disease was subsequently detected in many European countries [1,2].

Aspergilli are aerobes that are able to live and reproduce in the soil, on various organic substrates, and dead plant debris. Fungi also live as saprophytes on living plants, including on the stamens of flowers and

nectaries [4]. Pathogenic *aspergillus* species are introduced into hives by bees carrying nectar and pollen. Once inside the hive, these fungi can grow on honeycomb, bee bread, larvae, pupae, and adult bees at high humidity and temperatures [2,5]. The source of infection can also come from diseased or dead larvae and pupae, as well as from infected bees, honey, and bee bread that contain fungal spores. Hives and honeycomb that have been contaminated with fungal spores can also serve as a source of infection [6,7].

It should be noted that the spores of the pathogens have significant resistance in the external environment. Fungal spores remain viable for 4 to 15 years in empty hives, on honeycombs and beekeeping equipment, as well as in honey and bee bread [8,9].

Aspergillosis of bees is a zoonoanthroposis [10-14], and, therefore, strict personal hygiene measures should be followed when examining infected beehives and working with infected material. Beeswax collected from infected beehives should only be used for technical purposes, and pollen collection in these hives is prohibited. Honey collected from such hives should not be used for human consumption [5,11,15].

It is well known that the implementation of comprehensive measures to prevent and control the emergence and spread of infectious diseases, including bee Aspergillosis, through the use of disinfection is one of the key principles for ensuring sanitary and

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epidemiological well-being in apiaries in the country. In this context, the aim of this study is to search environmentally friendly disinfection agents with potential use in beekeeping and to evaluate their efficacy, both in laboratory settings and in practical applications in apiaries.

## 2 Method and materials

*Aspergillus flavus* (840-c/7), *Aspergillus niger* (843-c), and *Aspergillus fumigatus* (8/10) strains from the microorganism collection of the laboratory of mycotoxicology of the All-Russian Research Institute of Veterinary Sanitation, Hygiene and Ecology, as well as field strains isolated from pathological material at a private infected apiary in the Kaluga region, were used in this work. The collection strains were used as controls. After isolation and identification, field strains were used to carry out experiments both in laboratory and apiary conditions.

### 2.1 Light microscopy

The studies were carried out using the “crushed drop” method. To identify the pathogenic agent of aspergillosis, mycelium was scraped from the surface of the larvae. The material under study was placed on a glass slide in a drop of alcohol-glycerol, covered with a coverslip, and it was examined using the light microscope. A preliminary diagnosis of aspergillosis was made when fungal elements characteristic of the genus *Aspergillus* were detected in the pathological material.

### 2.2 Mycological studies

Diagnostic swabs or fragments of dead, mummified larva were placed on the surface of a nutrient medium in petri dishes. Czapek-Dox agar was prepared by melting it in a water bath prior to inoculation and cooling it to 40-45 °C. In order to suppress the accompanying bacterial flora, antibiotics were added to the cooled agar (50 000 units of penicillin and 100 000 units of streptomycin per liter of medium). The petri dishes were maintained in a thermostat at 25-27 °C and observed for a period of 14 days. Pure cultures were isolated by transferring the inoculum from the peripheral colonies in the primary cultures onto Czapek-Dox agar plates.

### 2.3 Laboratory and apiary trials

In the laboratory, artificially contaminated samples of wood, polystyrene foam, wax, metal, plastic, and textile measuring 10 × 10 cm were used. After a certain period of exposure (60 min and 180 min), samples were collected from the experimental and control surfaces using sterile cotton-gauze swabs that had been slightly moistened. These swabs were then placed in test tubes containing sterile water, which were used to inoculate Petri dishes. After 24 h and 48 h, the absence of growth of unwanted microorganisms was determined. The final results were observed after 7 days and 14 days. Based on

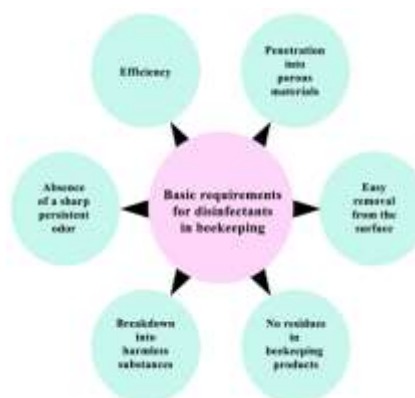
the presence or absence of test culture growth, the fungicidal and sporocidal efficacy of disinfectant was concluded. To ensure reliable results, each experiment was performed in triplicate.

In apiary experiments to test disinfection regimes for bee aspergillosis, 14 hives, 42 honeycomb frames and 26 items of beekeeping equipment were disinfected.

## 3 Results and discussion

The use of zootechnical measures and treatments for bee Aspergillosis is not a complete solution to the problem of hive sanitation. Therefore, the disinfection of apiaries is an essential component of a successful strategy for combating this disease.

Currently, both Russian-made and foreign preparations are permitted for use in disinfection in the field of beekeeping. Based on the active substance, these preparations can be classified into several chemical groups. Preparations containing active oxygen are widely used, as they best meet the fundamental requirements of this industry (Fig. 1).



**Fig. 1.** The basic requirements for disinfectants for use in beekeeping.

Based on a literature and patent search, the following oxygen-containing disinfectants were selected: hydrogen peroxide 37% (v/v%) for medical and veterinary use and “Dezinbak Super”, containing 98.6–99.4% (w/w%) peroxide compounds. These products meet the specified criteria and are manufactured in the Russian Federation and commercially available.

To identify clinical signs of aspergillosis in honeybees, the state of the larval stage of the brood was initially assessed through visual inspection. Typically, in broods affected by aspergillosis, larvae in the final stages of development (i.e., prepupae and pupae) of both worker bees and drones have died. Depending on the specific strain of *Aspergillus*, these broods may be covered with a variety of fungal growth, ranging from white to grey, yellow-green, or black. When examining bee colonies, it was noted that adult worker bees reduced their activity and lost the ability to fly. The belly of the diseased insects was enlarged and firm to the touch.

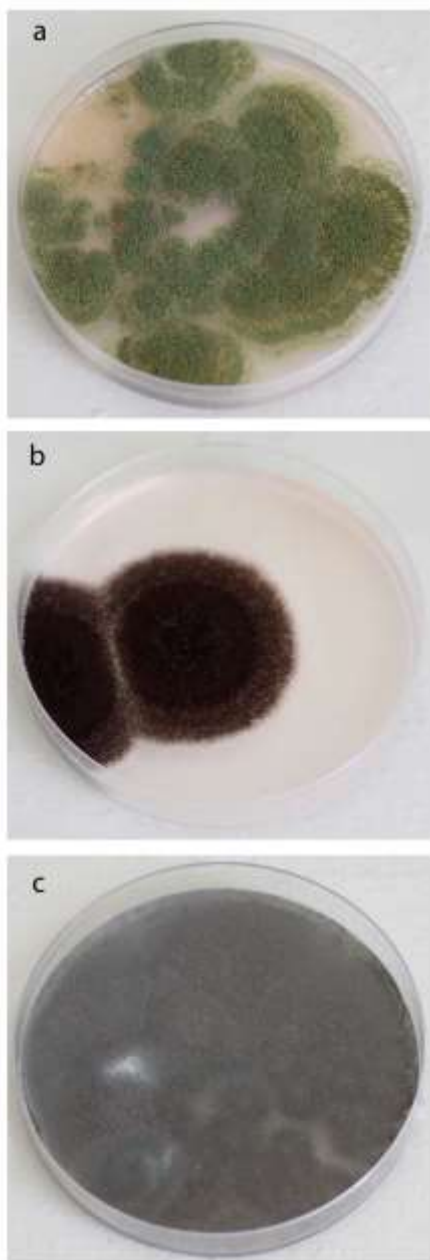
Data on cultural and morphological characteristics, as well as morphometric characteristics of fungi were obtained. This gave grounds to classify the isolated

isolates as certain species of pathogens. *A. flavus* was detected in 73%, *A. niger* in 18% and *A. fumigatus* in 9% of cases (Fig. 2).

concentration and exposure) were selected based on previous studies.

**Table 1.** The disinfectants efficacy against *A. flavus*

Disinfectants	Preparation concentration, %	Exposition, min	<i>Aspergillus flavus</i>						
			Wood	Polystyrene foam	Wax	Metal	Plastic	Textile	
Hydrogen peroxide	4	60	+	+	+	+	+	+	
		180	+	+	+	±	±	+	
	6	60	±	+	+	±	±	+	
		180	±	-	±	-	-	±	
	10	60	-	-	-	-	-	-	
		180	-	-	-	-	-	-	
“Dezinbak Super”	5	60	+	-	+	-	-	+	
		180	±	-	±	-	-	-	
	6	60	-	-	-	-	-	-	
		180	-	-	-	-	-	-	
	7	60	-	-	-	-	-	-	
		180	-	-	-	-	-	-	



**Fig. 2.** Isolated Aspergillus cultures: *A. flavus* (a), *A. niger* (b), *A. fumigatus* (c).

In 40% of cases, the causative agent of ascospheerosis, the fungus *Ascospheera apis fungus*, was found in conjunction with aspergilli. The concurrent growth of Aspergillus and Ascospheera fungal species was observed. The fungi were found to be non-antagonistic and did not inhibit the growth of each other, which explains the prevalence of the combined course of these mycoses.

The results of laboratory tests of oxygen-containing disinfectants are presented in tables 1-3 (“+” presence of fungal growth; “±” presence of partial growth; “-” absence of growth). The regimes (preparation

**Table 2.** The disinfectants efficacy against *A. fumigatus*

Disinfectants	Preparation concentration, %	Exposition, min	<i>Aspergillus fumigatus</i>						
			Wood	Polystyrene foam	Wax	Metal	Plastic	Textile	
Hydrogen peroxide	4	60	+	+	+	+	+	+	
		180	+	+	+	±	±	+	
	6	60	±	+	+	±	±	+	
		180	±	-	±	-	-	±	
	10	60	-	-	-	-	-	-	
		180	-	-	-	-	-	-	
“Dezinbak Super”	5	60	+	-	±	-	-	+	
		180	±	-	-	-	-	±	
	6	60	-	-	-	-	-	-	
		180	-	-	-	-	-	-	
	7	60	-	-	-	-	-	-	
		180	-	-	-	-	-	-	

**Table 3.** The disinfectants efficacy against *A. niger*

Disinfectants	Preparation concentration, %	Exposition, min	<i>Aspergillus niger</i>					
			Wood	Polystyrene foam	Wax	Metal	Plastic	Textile
Hydrogen peroxide	4	60	+	+	+	+	+	+
		180	+	+	+	±	±	+
	6	60	±	+	+	±	±	+
		180	±	-	±	-	-	±
	10	60	-	-	-	-	-	-
		180	-	-	-	-	-	-
“Dezinbak Super”	5	60	+	±	+	±	±	+
		180	±	-	±	-	-	±
	6	60	-	-	-	-	-	-
		180	-	-	-	-	-	-
	7	60	-	-	-	-	-	-
		180	-	-	-	-	-	-

In laboratory tests, the following regimes showed the best efficiency against all fungi species of the genus *Aspergillus* on all test objects: hydrogen peroxide at a concentration of 10% with an exposure of 60 and 180 min, “Dezinbak Super” at concentrations of 6 and 7% with an exposure of 60 and 180 min.

In addition, hydrogen peroxide was tested in the same concentrations (4, 6 and 10%), acidified with a solution of acetic acid to a content of 0.5%. The exposure time was 60 and 180 min (Tables 4-6).

**Table 4.** Hydrogen peroxide + 0.5% acetic acid efficacy against *A. flavus*

Disinfectant	Preparation concentration, %	Exposition, min	<i>Aspergillus flavus</i>					
			Wood	Polystyrene foam	Wax	Metal	Plastic	Textile
Hydrogen peroxide + 0.5% acetic acid	4	60	±	±	±	-	-	±
		180	±	-	-	-	-	-
	6	60	-	-	-	-	-	-
		180	-	-	-	-	-	-
	10	60	-	-	-	-	-	-
		180	-	-	-	-	-	-

**Table 5.** Hydrogen peroxide + 0.5% acetic acid efficacy against *A. fumigatus*

Disinfectant	Preparation concentration, %	Exposition, min	<i>Aspergillus fumigatus</i>					
			Wood	Polystyrene foam	Wax	Metal	Plastic	Textile
Hydrogen peroxide + 0.5% acetic acid	4	60	±	-	-	-	-	±
		180	±	-	-	-	-	±
	6	60	-	-	-	-	-	-
		180	-	-	-	-	-	-
	10	60	-	-	-	-	-	-
		180	-	-	-	-	-	-

**Table 6.** Hydrogen peroxide + 0.5% acetic acid efficacy against *A. niger*

Disinfectant	Preparation concentration, %	Exposition, min	<i>Aspergillus niger</i>					
			Wood	Polystyrene foam	Wax	Metal	Plastic	Textile
Hydrogen peroxide + 0.5% acetic acid	4	60	±	±	±	±	-	±
		180	±	-	-	-	-	±
	6	60	-	-	-	-	-	-
		180	-	-	-	-	-	-
	10	60	-	-	-	-	-	-
		180	-	-	-	-	-	-

As can be seen from the data presented in the tables, stable results were achieved with a 6% hydrogen peroxide content and an exposure time of 60 min.

The indicated regimes were tested in apiary trials, where they also demonstrated consistently high fungicidal and sporicidal activity.

## 4 Conclusion

Aspergillosis of bees is a zoonoanthroposis and when it is detected, effective veterinary and sanitary measures must be taken. Therefore, the search for disinfectants that meet industry requirements and the selection of treatment regimes for beekeeping objects are an urgent task for science and practice.

In this work, oxygen-containing preparations of “Dezinbak Super” and hydrogen peroxide were studied as disinfectants for the treatment of beekeeping objects

in laboratory and industrial conditions. The working concentrations and exposures of these drugs were determined, and regimens for their use were developed.

The data obtained provide grounds for recommending the tested preparations in the specified regimes for disinfection in case of aspergillosis of bees.

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