

# Use of environmentally safe preventive remedy against ascospheerosis

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**Abstract.** Death of bee families in many countries has led to a reduction in the number of honey bees, a reduction in their medicinal food products and pollination activity. Honey bees are susceptible to various diseases of an infectious nature, including a disease such as ascospheerosis caused by *Ascosphaera apis* fungus. The treatment of bee families against this disease with a preventive natural remedy, without the use of antibiotics and chemicals, is included in the concept of organic bee-farming and leads to a decrease in the percentage of affected families. Two groups of bee families treated with prophylactic compounds from natural raw materials were analyzed in the course of the research: experimental group № 1 (garlic, wormwood with vitamin C); experimental group № 2 (garlic and 5% iodine solution). Laboratory and field studies confirm the greatest effectiveness of garlic and iodine composition. There is a reduction in infection of bee brood due to the active substance allicin, which carries antimicrobial properties and, accordingly, increases the number of working flying bees.

## 1 Introduction

Honey bee is one of the most important types of insects for the full functioning of many terrestrial ecosystems. It provides not only valuable and medicinal food, but is also a pollinator for many plants [3, 9].

However, bee-farming is influenced by various factors: abiotic, biotic and anthropogenic [1, 6, 7]. In recent years, the annual decline in bee families occurs under the influence of various diseases (viruses, bacteria, ticks) and changes in environmental conditions.

The collapse phenomenon of bee family/colony, Colony Collapse Disorder - CCD had been widespread [5, 8].

Focal outbreaks of diseases of varroaosis, nosematosis and ascospheerosis lead to a decrease in insect immunity, and accordingly, to a decrease in the number of families and the productivity of the industry as a whole.

The search for environmentally safe drugs for treating bee colonies against various diseases is becoming an urgent task today.

The presence of a variety of infectious and invasive diseases was identified during studies in the Udmurt Republic to study the background incidence of bee families: varroosis, nosematosis, acarapidosis, European and American foulbrood and ascospheerosis. The largest percentage of diseased bee colonies, that was 77.5% were affected by *Ascosphaera apis* fungus [4, 10].

This bee brood disease is one of the most common and dangerous infections for bees, from which apiaries suffer significant damage. Affecting brood at 3-4 days of age, ascospheerosis leads to a weakening of a bee family until its death [2].

In this regard, the use of natural medicinal substances has several advantages over chemical preparations and acts on animal organism in a complex without disturbing basic physiological processes, stimulating the resistance of bees.

## 2 Materials and research methods

The purpose of this study is to determine the impact of an environmentally safe remedy based on natural raw materials against ascospheerosis of bee families on their vital activity and productivity.

The testing of two sets of preventive remedies composed of natural components was carried out during the research:

Experimental group № 1. The following components were included in the first version of the preventive remedy: garlic, wormwood with ascorbic acid. All surfaces in the bee family nest were treated with the obtained solution by aerosol spraying in the spring at a rate of 200 ml per family.

Experimental group № 2. The environmentally safe product included such natural ingredients as: garlic and

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5% iodine solution. The active substance of this remedy is allicin, which has antifungal and antimicrobial effects. Allicin was obtained by grinding garlic and creating an aqueous extract, this solution is the basis for processing bee families. In addition to allicin, the remedy contains phytoncid, the presence of which provides a reduction in fungal diseases.

The addition of a 5% iodine solution enhances the antimicrobial effect and dissimilation processes (decomposition of complex organic substances into simpler ones), favorably affects lipid and protein metabolism. The use of this combination during prophylactic treatments of bee families has not previously been recorded. All surfaces in the bee family nest are treated by the resulting solution with aerosol spraying method in spring at a rate of 200 ml per family.

The studies on the development of bee family brood and their honey productivity were carried out during two summer field seasons of 2016-2017 in accordance with the developed methods in bee-farming [8].

Bee families without treatment with prophylactic and therapeutic drugs against ascospheerosis were used as a control group.

Laboratory studies were conducted in the laboratory of FSBEI HE Izhevsk State Agricultural Academy, *Ascospheera apis* fungus was grown according to the method of O.F. Grobov (1989).

### 3 Research results

During laboratory studies, when processing with the obtained solution consisting of garlic and iodine components, an insignificant demonstration of colony growth in the treated Petri dish was revealed, in the amount of 5%, when using an experimental solution (garlic, wormwood and ascorbic acid), the presence of fungus was detected in the amount of 14%, while a continuous growth of the fungus over 80% was observed in untreated Petri dish.

Field studies were conducted in an apiary in Zavyalovsky district of the Udmurt Republic. 3 groups of bee families were formed for the experiment by the method of pair-analogues, in accordance with the approved methodology.

In this case, the construction of hives (16-frame), the queen bee age of not older than 2 years and the amount of strength during spring revision (6 bee spaces) were taken into account.

Each group included 10 families according to the scheme: control group - without treatment with a complex remedy, experimental group № 1 – brood and bee irrigation with a preventive remedy (garlic+wormwood+vitamin C), experimental group № 2 – brood and bee irrigation with a preventive remedy (garlic +5% iodine solution).

The experimental groups were treated by irrigation in the first spring inspection of bees when the air temperature reached + 14-15°C.

Bee families with the same number of brood were selected for the experiment, without the presence of visible signs of ascospheerosis. After 12 days (growth

period of the unsealed brood of bees) signs of ascospheerosis were checked. This disease manifests itself in adverse climatic conditions, that is, high humidity and return cold. Since the Udmurt Republic is characterized by an unstable spring, this disease manifests itself quite often in apiaries (Table 1).

Table 1. Bee resistance studies

Indicator	Group		
	Control	Experim ntal №1	Experime ntal № 2
affected larvae presence, %	25.9	15.7	2.1

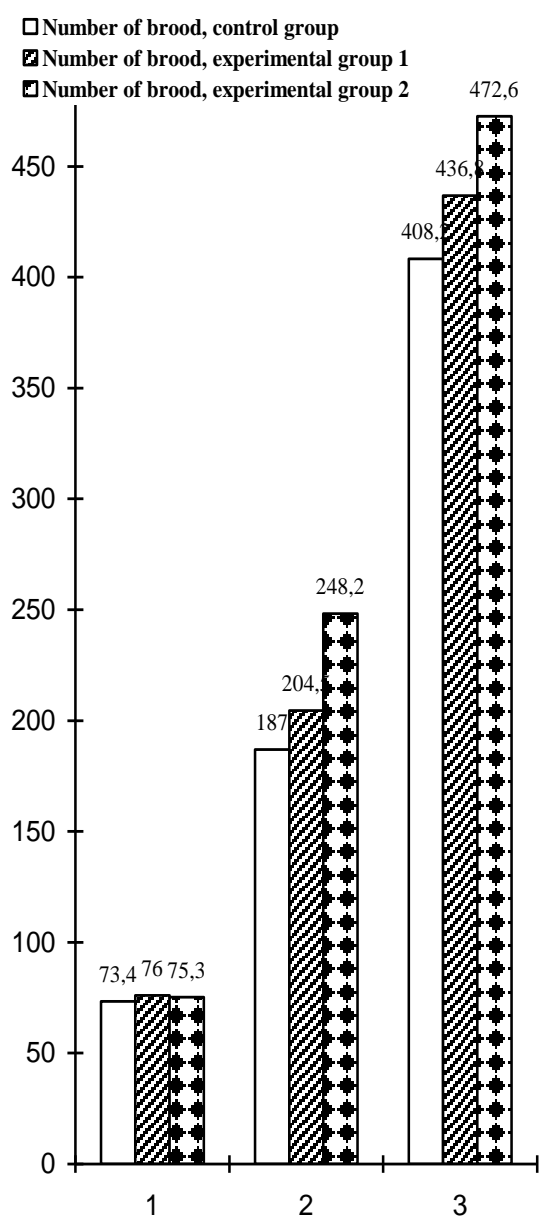


Fig. 1. Bee family growth in the spring-summer period

Resistance of bee families was analyzed during field studies. After treatment in the experimental group № 2,

the presence of ascospores signs was not practically observed (2.1%), this indicator was higher by 13.6% in experimental group № 1, signs of disease were present in an amount of 25.9% in the control group.

The analysis of the growth of brood number was carried out three times every 21 days in accordance with the growth period of the working bee (Fig. 1).

The following regularity was revealed in the analyzed summer period. A difference of 17.5 hundred cells was observed between the control and experimental group № 1 to the second measurement for the brood number and between the control group and experimental group № 2 in the amount of 61.2 hundreds of cells or 32.7%, and during the third measurement, the difference was 25.9 and 64.4 hundreds of cells, respectively.

An analysis of growth indicators of bee families over the summer period revealed the most effective use of a set of components of garlic and iodine solution for processing bee colonies against ascospores, since the families of experimental group № 2, where this agent was used, had increased 472.6 hundreds of cells at the third measurement time with egg-laying production of the queen bee equal to 2250.1 eggs per day, which is as close as possible to the physiological possibility of egg-laying production of queen bees at their peak of egg laying in the amount of 2500 eggs per day.

First of all, honey productivity of bee families is one of the main economically useful indicators. The amount of honey and wax productivity is shown in Table 2.

**Table 2.** Honey and wax productivity (per one bee family, kg)

Indicator	Control group	Experimental group № 1	Experimental group № 2
	$\bar{X} \pm m_x$	$\bar{X} \pm m_x$	$\bar{X} \pm m_x$
Marketable honey, kg	26.1±2.41	34.5±2.45	39.2±1.77* **
Gross honey, kg	60.4±2.36	63.7±3.65	73.0±1.72* **

Note: \*\*\*P<0.001

39.2 kg of marketable honey was received in the summer period of 2017 from experimental group № 2, which is 13.1 kg more than in the control group and 73.0 kg of gross honey, which is 12.6 kg more. The number of rebuilt sheets of unrefined beeswax in the experimental group № 2 was 4.8 pcs, which are 0.4 sheets more than in the control.

In recent years, mankind around the world has been more painstaking in choosing food products. In connection with this, the requirements for them are being tightened. Increasingly, we hear about organic products, which means that they were produced without the use of chemistry and antibiotics.

In this regard, we also conducted studies of the quality of honey products.

The aroma and taste of all samples are pleasant, and they have no extraneous odors and aftertastes.

The humidity of honey is influenced by many factors: suitable conditions during the honey harvest

season, the degree of ripeness of honey, the ratio of sugars, nectar excretion, storage conditions and type of packaging. The weight fraction of water in the analyzed samples corresponds to GOST (no more than 21%). In experimental group No. 1, it was 19.9%, and in experimental group No. 2 - 18.6%.

The diastase number indicates the naturalness of honey. The diastase enzyme catalyzes the breakdown of starch into maltose disaccharide, which subsequently breaks down with the release of glucose. Requirements for the diastase number in Russia are at least 7 Gaulté points. In the analyzed period, the diastase number in the experimental group No. 1 was 20.25, and in the experimental group No. 2 it was 18.63 Gaulté points. Sugars make up the bulk of honey. The content of reducing sugars corresponded to the standard (at least 82%) in the honey sample in the experimental group No. 1 (83.73%) and in the sample in the experimental group No. 2 (86.50%).

The acidity of honey is a sign of its naturalness and quality. Increased acidity is a sign of acidification of honey and the accumulation of acetic acid in it, or it confirms that this honey is artificial. Acidity less than 1.0 also indicates the unnaturalness of honey.

**Table 3.** Organoleptic and physico-chemical characteristics of the studied honey

Indicator	GOST 54644-2011	Year	
		2016	2017
Aroma	Pleasant, from mild to strong, without extraneous odor	Pleasant, strong, without extraneous odor	Pleasant, strong, without extraneous odor
Taste	Sweet, pleasant without any aftertaste	Sweet, pleasant without any aftertaste	Sweet, pleasant without any aftertaste
Water weight fraction, %, no more than	21.0	19.9±0.20	18.6±0.32
Diastase number, Gaulté points, no less than	7.0	20.25±1.32	18.63±1.84
Total acidity, cm <sup>3</sup> NaOH, no more than	4.0	1.50±0.084	2.41±0.69
Weight fraction of reducing sugars, % no less than	82.0	83.73±0.24	86.50±0.37
Qualitative reaction to hydroxymethyl furfural	Negative	Negative	Negative
Signs of fermentation	Not allowed	No signs	No signs
Mechanical impurities	Not allowed	No impurities	No impurities

The acidity of honey in these samples for the study period was 1.50 cm<sup>3</sup> (experimental group No. 1) and 2.41 cm<sup>3</sup> (experimental group No. 2) (according to the norm, not more than 4.0). The qualitative reaction of honey to oxymethylfurfural for the studied years was negative. There were also no mechanical impurities or signs of fermentation in the samples for the analyzed period, which corresponds to the sanitary requirements for the quality of honey.

Thus, studies have shown that all samples comply with GOST 54644-2011 "Natural honey. Technical conditions."

At the same time, bee products are exposed to toxic pollution as well as soil, crop and livestock products.

Currently, it has been convincingly proved that the ecological purity of honey products depends on a whole range of factors:

- the level of environmental pollution: soil, water and air, i.e. the content of heavy elements and radionuclides;
- the species composition of honey plants and pollen plants and soil type;
- the type of honey products (honey, wax, propolis, pollen, royal jelly, bee venom, etc.);
- compliance with sanitary and veterinary requirements in the apiary.

As a result, studies were carried out on the presence of heavy metals and radionuclides in honey samples (table 4).

Samples of the study period revealed slight traces of mercury (Hg) (less than 0.004 mg/kg). Other indicators shown in Table 4 also did not exceed the maximum permissible concentrations.

**Table 4.** The average content of heavy metals and radio nuclides in honey samples

Indicator	Maximum permissible concentration	2016 study	2017 study
Cadmium (Cd), mg/kg	0.05	0.003	0.005
Lead (Pb), mg/kg	1.0	0.109	0.117
Arsenic (As), mg/kg	0.5	0.074	0.080
Cesium-137 (Cs), Bq/kg	100	0.365	0.767
Strontium-90 (Sr), Bq/kg	80	0.854	0.933

The table shows the radionuclides of a long decay period: cesium-137 and strontium-90.

Thus, studies have shown that all samples comply with GOST 54644-2011 "Natural honey. Technical conditions." The quality of honey meets sanitary standards (SanPiN 2.3.2.1078-01) for all indicators.

## 4 Conclusion and recommendations

Laboratory and field studies confirm the greatest effectiveness of garlic and iodine composition. Due to the active substance allicin, which carries antimicrobial properties, there is a reduction in bee brood infection and, accordingly, the number of working flying bees increases. This indicator was higher by 20.0% (2016) and 15.8% (2017) in the experimental group № 2 than in the control group to the main honey collection. Accordingly, more working power leads to more intensive collection of nectar. Bee families in the experimental group № 2 collected more marketable honey by 35.1% in 2016 and 50.2% in 2017 compared to the control group.

Thus, the preventive remedy use consisting of natural components is not only environmentally safe and does not remain in the final product - honey, but also contributes to the colony growth of bee families and honey collection for marketable and gross productivity.

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