The effectiveness of Tolkoks 2.5% medication in treating nosematosis in bees (Uzbekistan)

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Abstract. This article presents the results of testing the medication Tolkoks 2.5% as a therapeutic agent in honeybee colonies infected with nosematosis under natural beekeeping conditions. The medication was prepared by adding 0.25 ml of Tolkoks 2.5% to 1 liter of sugar syrup and administered once at a dose of 250 g for 12 hours. Scientific results obtained from experiments and laboratory research are described in this article.

1 Introduction

The relevance of the topic. Beekeeping plays a significant role in implementing programs to ensure the quality and safety of food products. Currently, there is a pressing need for the use of new medications and the conduct of scientific experiments aimed at preventing and treating bee diseases within bee colonies.

As mentioned earlier, Uzbekistan has a favorable climate for beekeeping, but sometimes, due to unfavorable weather conditions, when feeding bees with poor-quality food, non-contagious, invasive, and infectious diseases arise. Nosematosis in bees is one of these diseases. The disease occurs mainly in early spring and late autumn with high humidity, spreads to bee colonies, and causes great damage to beekeeping farms.

In the last five years, beekeeping farms around the world have seen the death of bees due to various environmental factors. It is known that any disease weakens the bee colony, especially it has a great impact on the work of bees, leads to a decrease in honey productivity and an increase in the price of honey.

Nosematosis of bees belongs to the group of quarantine diseases. The disease is more often registered in the spring and, in some cases, in the autumn. Clinical symptoms are restlessness in bees and severe diarrhea. In affected colonies, partial or massive bee deaths are observed Nosematosis is currently a frequently discussed honey bee disease caused by two types of Microsporidia: Nosema apis and Nosema ceranae. Nosematosis as an intestinal disease caused by these species is one of the main factors associated with the weakening and loss of hives, with none of the stressors acting in isolation and all having an important synergistic or additive effect on the occurrence of parasitic infection. The most important factors are exposure to pesticides and nutritional stress, both worsening the immune response. Honey bees Apis mellifera become more susceptible to parasites and

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subsequently the disease manifests itself. Choosing the right laboratory diagnostics is important to determine the prevalence of both species (1-photo) [1, 2] .

It is known that this extremely dangerous disease of the honey-bees (Apis mellifera) is caused by a spore amoeba called Nosema apis. The main period of the disease is in spring, although the disease itself can occur at other times, too. The content of the rectum of the healthy winter bees increases only slowly in the first half of the winter. During the last third of the winter a sudden increase occurs, because the commenced brood raising forces the otherwise healthy bees to increase their metabolism. The content of the bees infected with nosema increases gradually, because the water content of the intestine increases, this adds to the otherwise tense content of the rectum and consequently the ill start defecating within the hive. The excrements get on the bees, on the walls of the cells, on the cell-caps, pollen, and on the honey. The healthy members of the colony get infected due to their cleaning instinct. The cleaning bees suck the thin excrement and they lick the dried excrement, too, after previously moistening it. During this activity the bees get infected with huge amounts of Nosema apis spores [8].

According to the researchers, infection of young bees with nozema spores can cause digestive problems for the rest of the bee's life. These bees do not normally produce brood food secretions from the hypopharyngeal glands and often skip the brood-rearing stage of their lives and become bees when they are young. when queen bees are infected with nosematosis, their lifespan is shortened and they stop laying eggs. These effects lead to a decrease in health and productivity of the bee colony, resulting in the death of the bee colony.The pathogen of the disease is the unicellular organism *Nozema*. There are two species of pathogens: *Nozema apis* and *Nozema ceranae microsporidia*, which parasitize the epithelial cells of the midgut of bees [3].

In 2011, it was first established in Russia that up to 10% of *Nozema ceranae* spores are common in Siberian apiaries. 7–14 days after bees were infected with *Nosema* spores, they developed weakness, decreased their reaction to external stimulation, their abdomen became swollen, their wings turned out, and paresis and paralysis of the insect's limbs were observed Infection of bees with nosematosis depends on the age of the bees. One-day mortality of bees was recorded at a dose of 200,000 spores per bee. Within 3 days, after 15 days - after 5-7 days, the longest survival was recorded for 3-7 days. Infestation of worker bees occurs when they are fed by 2-6 day old worker bees, which also participate in the cleaning of honey molds. The fertilized queen bee is infested by worker bees for 6-13 days. In this way, the disease spreads to the bees in the hive [4].

Currently, many drugs are available for the treatment of nosematosis. One of them is Fumagilin-B (manufactured by Medivet Pharmaceuticals Ltd., Canada), which has undergone production testing and is used for the prevention and treatment of nosematosis in bees. It effects by eliminating DNA replication in microsporidia by delaying and destroying the vegetative stages of microsporidia of the genus *Nosema* [5].

Many researchers nozematous fumagillin drug manufactured by Sanofi-Chinoin Drug Manufacturing Company Budapest, Hungary Fumagillin DCH pulvis is sold in bottles of 20 g. One bottle contains 0.5 g of pure active substance. According to the instructions for use, the content of one bottle is enough to treat 10 lightly or 5 heavily infected colonies. Before use, the contents of the bottle should be dissolved in 0.5 liters of warm water, then mixed with 5 liters of sugar syrup in a ratio of 1:1, cooled to 40 °C, and then diluted to 25 °C. sugar syrup. Sick colonies are fed with 0.5 liters of mixture every day [9-10].

In addition, as a result of scientific research conducted by scientists, a comparative analysis of the treatment of bee colonies in hives with the drugs "Nosemacid", "Nozevir" and "Nozemat" was carried out. They were used in the treatment of bees infected with nosematosis in beekeeping farms in Samara region of Russia. In the treatment of the disease, on the basis of the experiments carried out by scientists, Nozemat was given at a

concentration of 0.025% (0.5 ml for 2 liters of syrup) for 10 days. On the 11th-12th days, the presence of N. apis spores in the feces of sick insects was checked in the laboratory, and there were no Nozemat spores. has been determined. Adding Nozemat to the syrup to prevent nosematosis is said to be a cure for the disease in bees. To prevent nosematosis, it is necessary to provide bee families with sufficient protein food during their active life. A strong family with high-quality honey should be left in the village with a large number of young bees. Weak families, that is, families that could not complete their development in the summer, unite, settle in one nest and wrap themselves in warmth. Disinfection events are held every year [6, 11].

According to scientists, the liquid feed additive for bees produced by "Save the Bees" for the prevention of nosematosis based on the live bacteria Bacillus subtilis contains at least $1x10^8$ cells of living bacteria in 1 ml of the drug, as well as their metabolic products: polypeptide antibiotics, enzymes, amino acids, and other biological active substances that increase immunity and functional activity of the intestines. Due to the stability of the intestinal function and immune system of bees, immunity against diseases is formed [7].

Purpose of the research. Study of the effectiveness of the therapeutic and nosemicidal action of Tolkoks 2.5% (25 mg of the active substance Toltrazuril in 1 ml) on bees infected with nosematosis in beekeeping.

2 Materials and methods

Research work took place in the Opta-Tech laboratory, organized by the Poland Embassy in Uzbekistan, at the department of "Diseases of Poultry, Fish, Bees, and Fur Animals" of the Samarkand State University of Veterinary Medicine, Livestock, and Biotechnologies, in the laboratory of the innovative project PZ-2020123121 of the Ministry of Innovative Development of the Republic of Uzbekistan, control work was carried out under a MIC D30 microscope, and experiments were carried out on bees of the "Karpat" breed in the private beekeeping farm "Ravonak" in Samarkand district of Samarkand region, Uzbekistan. In scientific experiments, *Nosema* spores were observed in the laboratory under a microscope using the "crushed drop" method, the number of *Nosema* spores was counted using a Goryaev camera, and the level of infestation was assessed using a 4-point system (based on the result: low level of infestation: 10 spores in the field of view; medium: up to 100; strong: up to 1000; very strong: more than 1000; more than 1000 Nosema spores).

3 Results and Discussion

Bee colonies affected by nosematosis on a beekeeping farm were selected for study by observation. For the study, groups were prepared from each bee colony affected by nosematosis: one experimental and two control. In the hives of the experimental and control groups, 4300 worker bees and 1 queen were placed per 5 frames and medium-strong bee colonies. As a therapeutic medication, we used Tolkoks 2.5% (manufactured by PE "O.L.KAR-AgroZooVet-Service" Ukraine; the medication contains 25 mg of the active substance Toltarazuril in 1 ml, issued in a transparent, odorless, liquid glass ampoule). As a result of group scientific experiments conducted in the laboratory, the toxic dose and therapeutic dose of the medication were determined. The medication syrup prepared in the laboratory was prepared in the same way on the bee farm. The bees of the experimental and control groups were not given any food 1 hour before the medication was administered. Bees in the first experimental group were fed 250 g of Tolkoks 2.5% syrup once for 12 hours. The medication Fumagilin-B (manufactured by Medivet Pharmaceuticals Ltd., Canada), containing the active substance fumagilin bicyclohexalamine, was used for bees

infected with nosematosis in the second control group. According to the instructions, 2 g of the medication were added to 1 liter of sugar syrup; 250 g of this mixture was given once, and observations were made. Bees in the third control group infected with nosematosis were given only sugar syrup (Table 1).

Table 1. Dose of Tolkoks 2.5% and Fumagilin-B medication, which were given to the hives of experimental and control groups (n>30).

No.	Group name	Average quantity of bees in 5 hive frames	Medication name	Dose of the medication and sugar syrup volume	Dose of the syrup mixture
1	Experimental	4324	Tolkoks 2.5%	0.25 ml + 1L	250 g
2	Control	4315	Fumagilin-B	2 g + 1L	250 g
3	Control	4332	Sugar syrup	-----	------

Samples of living and dead worker bees, taken separately from the bee colonies of the experimental and control groups, were examined in the laboratory using the above methods.

In the conducted scientific studies, based on observations and results obtained in laboratory conditions, the effectiveness of a single administration of Tolkoks 2.5% to the bees of the first experimental group and to the bees of the control groups was determined (Table 2).

Table 2. Therapeutic effectiveness of Tolkoks 2.5% medication for bees infected with nosematosis, and the results of laboratory analysis (n>30).

No.	Group name	Medication name	Bees' quantity in groups	Dose of the syrup mixture	Results	
					Number of death bees	%
1	Experimental	Tolkoks 2.5%	4324	250 g	92	2.2
2	Control	Fumagilin-B	4315	250 g	176	4.0
3	Control	Sugar syrup	4332	250 g	217	5.0

On the 3rd day after administering the medication, the bees of the first experimental group stopped having diarrhea; there was no creeping movement along the wall of the hive; the number of dead worker bees around the hole at the entrance and exit of the hive was minimal; and the bees in the group recovered. Samples were examined in the laboratory for the presence of Nosema spores at a magnification of WF10 x 100/1.25 on a MIC D30 trinicular microscope. When assessed in a 4-cell system, it was found that the absence of spores in samples taken from the bees of the experimental group does not indicate the presence of invasion. The bees of the second control group were fed the medication Fumagilin-B according to the instructions. The group's bees indicated clinical signs of nosematosis: diarrhea, bloating of the abdomen, and a humming sound at the bottom of the hive. The instructions recommend adding the medication to the bees' food for 21 days. The results of laboratory analysis and a 3-point assessment showed that the bees in the control group had a moderate degree of affect by the disease. Comparing the results obtained in the experimental and control groups, the experiment found that the effect of 0.25 ml of Tolkoks 2.5% was higher.

4 Conclusion

When treating the nosematosis disease of bees by mixing 0.25 ml of Tolkoks 2.5% with 1 liter of sugar syrup and feeding the bee colony once for 12 hours, it was found that the

nosematicidal effect of the medication is high and at the same time completely harmless to bees and their offspring. The treatment of bee colonies with Tolkoks 2.5% should be carried out in the spring and after the summer, no later than a month before the start of the main honey harvest. In the autumn period, it is done based on the results of pumping out honey.

In conclusion, it should be noted that in order to increase the sustainability of economic growth in beekeeping, it is advisable to implement veterinary and sanitary measures and mechanisms within a seasonal and planned framework.

Fig. 1. A family of bees affected by nosematosis.

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