

Study of effectiveness of lactic acid at varroatosis in the apiaries of Tyumen region, Russia

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The study objective is to study the effectiveness of lactic acid in varroatosis and its impact on the development of bee colonies under conditions of the South of Tyumen region. The acaricidal effectiveness of lactic acid was studied in October 2019 using one method in two experiments in 30 broodless bee colonies kept in two apiaries, where bee colonies were divided into 2 groups (experimental - 10 colonies and control groups of 5 colonies each). For treatment of bee colonies from the experimental group, frames with bees were removed from the hives in turn, which were sprayed with a 15.0% aqueous solution of lactic acid in the volume of 5 ml on each side of the honeycomb. Bee colonies were treated twice with an interval of 5 days. The bees in the control group were not treated. Dead mites were registered on a daily basis in all groups within 5 days after each treatment, for these reasons laminated cardboard sheets were placed on the bottom of the hives, which then were taken out and the number of fallen ectoparasites was recorded. Studies on the impact of lactic acid on the development of bee colonies were conducted in 20 brood bee colonies in the period from May to July 2020. It has been found that the effectiveness of treatment of experimental bee colonies with the drug was $90.0 \pm 2.1\%$ (apiary No 1) and $7.8 \pm 1.9\%$ (apiary No 2). At the same time, the maximum death of mites in colonies was observed during the first day after treatment. In the control groups, the number of mites decreased by $10.9 \pm 0.8\%$ and $11.6 \pm 1.0\%$, respectively. It has been established that the treatment of bees with an aqueous solution of lactic acid in the above concentration, dose and multiplicity did not have a negative impact on the development of bee colonies, their strength and food supply, and no deaths of bees and queens were detected during the observation period.

Keywords: bee colonies, varroatosis, lactic acid, effectiveness, Tyumen region, Russia

Introduction

Diseases and pests of bees have harmful effect on the world beekeeping. The pathogenic agent of varroatosis, the mite *Varroa destructor*, is a vector of many pathogens of bacterial, viral and fungal infections, by contributing to mixed infections and infestations and the death of bee colonies around the world (Fig. 1).



Fig.re 1. Mites *V. destructor* on larvae and pupae of bees *A. mellifera*

The widespread use of medicines based on pyrethroids, organophosphates, and formamidines for the treatment of bee colonies against varroatosis has led to the fact that mites have evolved resistance to most acaricides containing amitraz,

coumafos, fluvalinate and flumethrin as active substances, which also accumulate in bee products (Milani, 1999; Wallher, 1999; Sammataro et al., 2005; Tihelka, 2018; Morgan et al., 2020). Therefore, many scientists are searching for highly effective, environmentally friendly means, including organic acids, to control the *Varroa mites* in bee colonies. Formic (Rashid et al., 2012; Pietropaoli & Formato, 2018), oxalic (Rashid et al., 2012; Adjlane et al., 2016; Maggi et al., 2017) and lactic acids (Rosenkranz et al., 2010; Morgan et al., 2020) have been studied and proposed to control varroaosis. The studies of acaricidal activity of lactic acid in varroaosis in different countries have shown different effectiveness of the drug (from 80 to 99.8 %), depending on the concentration of the active ingredient, dose, physiological state of bee colonies, season and climate. Based on the data obtained, most researchers recommended that lactic acid be used in order to treat bees against varroaosis in small apiaries due to the labour intensity of using the drug (Kraus & Berg, 1994; Colin, 1997; Wallner, 2003; Rosenkranz et al., 2010; Girişgin & Aydin, 2010; Galatyuk et al., 2015). In Soviet Union, the study of effectiveness of lactic acid against varroaosis was conducted at the All-Russian Research Institute of Experimental Veterinary Medicine of veterinary hygiene in the nineties of the 20th century. The therapeutic effectiveness of 5, 10, 15, and 20% aqueous solution of the drug on bee colonies, its effect on the biochemical and physiological indicators of insects in laboratory and apiary environment, and toxicity to bees and warm-blooded animals were studied. As a result of studies, a 10% aqueous solution of the drug was recommended for the treatment of bees, the therapeutic effectiveness of which was $80.1 \pm 1.9\%$ when treating brood bee colonies (Lugansky et al., 1987). In 1989, the Main Veterinary Department of the USSR Ministry of Agriculture registered the application of lactic acid for treatment of bees against varroaosis. The adopted document recommended to use the drug four times during the active beekeeping season: in the spring – after sanitation of the hives twice with an interval of 7 days, and, in the same way, in the summer after removal of honey combs from the hives. The treatment regimen provided for extracting bee frames from hives and spraying them with a 10.0% aqueous solution of lactic acid in the amount of 5-6 ml on each side of the frame at a temperature not lower than 15 °C (Kotova et al., 1992). Introduction of specific acaricides based on the above-mentioned active substances on the Russian market reduced studies on lactic acid, which led to the lack of objective data on the effectiveness of the drug in different climatic and natural conditions of Russia. In this regard, the objective of our study was to study the acaricidal activity of lactic acid in varroaosis and its impact on the development of bee colonies in apiaries of Tyumen region.



Fig. 2. Tyumen Region Map

Materials and methods

The effectiveness of lactic acid for treating bee colonies against varroaosis was studied in two experiments using one method in October 2019 on 30 broodless bee colonies of two apiaries located in Tyumensky District (Fig. 2). Bees were kept in 12-frame hives with removable bottom board. Colonies strength averaged 10.0 ± 0.5 frames. During the study period, the average air temperature was 5.9-14.6 °C, the daytime temperature -7.3-19.0 °C and the night temperature -7.0-14.3 °C. In both experiments, bee colonies were divided into 2 groups (experimental - 10 colonies and control group of 5 colonies each). For treatment of bee colonies from the experimental group, frames with bees were removed from the hives in turn, which were sprayed with a 15.0% aqueous solution of lactic acid in the volume of 5 ml on each side of the honeycomb (Fig. 3). Bee colonies were treated twice with an interval of 5 days. The bees in the control group were not treated. Dead mites were registered on a daily basis in all groups within 5 days after each treatment, for these reasons laminated cardboard sheets were placed on the bottom of the hives, which then were taken out and the number of fallen ectoparasites was recorded. After that, the experimental and control colonies were treated twice with bipin (12.5% amitraz emulsion concentrate) according to the instructions for use. The effectiveness of treatment was determined according to the formula:

$$\text{Effectiveness of lactic acid, \%} = \frac{\text{The number of dead mites after treatment with lactic acid}}{\text{The number of dead mites after treatment with lactic acid and bipin}} \times 100$$



Fig. 3. Treatment of bee colonies with lactic acid

To conduct study of effect of lactic acid on the development of bee colonies, in May 2020, 20 bee colonies were selected, which were divided into two groups – an experimental and a control group of 10 colonies each. Bee colonies were kept in 16-frame hives. Groups were formed by analogy, taking into account the age of queens, the colonies strength, food supply, and low (<1.0%) infestation with *Varroa destructor* mites. Bee colonies from the experimental group were treated with 15.0% lactic acid solution by removing from the hives and spraying bee frames in a volume of 5 ml on each side of the honeycomb twice at intervals of 5 days. Control colonies were not treated. For three months after treatment, all bee colonies were controlled every 2 weeks, the number of bee frames was taken into account, and the number of sealed brood was determined using a frame-grid with squares of 25 cm², including 100 bee cells. Frames with honey and bee bread were weighed. The results of all experiments were analyzed using the One-Way ANOVA.

Results and Discussion

The study found that double treatment (at 5 days intervals) of broodless bee colonies with 15.0% aqueous solution of lactic acid by spraying frames with bees in the volume of 5 ml on each side of the honeycomb reduced the number of *Varroa mites* by 90.0±2.1% (94.1-86.3%, apiary No 1) and 87.8±1.9% (89.9-84.8%, apiary No 2), respectively. At the same time, the maximum death of mites in colonies was observed during the first day after treatment. In the control group (apiary No 1), mite mortality during the observation period corresponded to 10.9±0.8% and in apiary No 2 – 11.6±1.0%. The results of the study are presented in tables 1 and 2.

Table 1. Effectiveness of lactic acid in varroatosis, apiary No 1.

Groups, No. of bee colonies	Number of dead mites after the first treatment	Number of dead mites after second treatment	Number of dead mites after treatment (total)	Number of dead mites after treatment with bipin	Effectiveness of the treatment (%)	Average effectiveness M±m (%)
Experimental (lactic acid)						
1	301	244	545	49	91.7	90.0±2.1
2	282	200	482	51	90.4	
3	331	211	542	60	90.3	
4	295	181	476	56	89.5	
5	224	151	375	51	88.0	
6	256	181	437	46	90.4	
7	224	148	372	38	90.7	
8	310	185	495	61	89.0	
9	226	154	380	60	86.3	
10	230	173	403	25	94.1	
Control (without treatment)						
11	33	30	63	479	12.0	10.9±0.8
12	28	30	58	437	11.7	
13	30	29	59	560	9.5	
14	25	35	60	497	10.8	
15	34	24	58	498	10.4	

Table 2. Effectiveness of treatment of bee colonies with lactic acid, apiary No 2

Groups, No. of bee colonies	Number of dead mites after the first treatment	Number of dead mites after second treatment	The number of dead mites after treatment (total)	Number of dead mites after treatment with bipin	Effectiveness of the treatment (%)	Average effectiveness M±m (%)
Experimental (lactic acid)						
1	251	213	464	53	89.7	87.8±1.9
2	235	157	392	61	86.5	
3	331	221	552	69	88.9	
4	294	196	490	89	84.6	
5	279	231	510	67	88.4	
6	213	189	402	72	84.8	
7	275	199	474	57	89.3	
8	309	215	524	69	88.3	
9	317	213	530	59	89.9	
10	280	203	483	67	87.8	
Control (without treatment)						
11	33	36	69	513	11.8	11.6±1.0
12	43	30	73	515	12.4	
13	37	34	71	491	12.6	
14	31	36	67	543	10.9	
15	32	33	65	570	10.2	

After treatment no dead queens was recorded when controlling bee colonies of the control and experimental groups. Studies of the impact of lactic acid on the development of bee colonies showed that during the observation period, bee colonies from the experimental and control groups did not differ in strength ($t=0.50$), number of brood ($t=1.20$) and feed ($t=0.94$) at $P<0.05$ as of the registration date of 05.07.2020 (table 3)

Table 3. Condition of bee colonies after treatment with lactic acid (M±m)

Date of registration	Name of group					
	Experimental			Control		
	Number of frames with bees (pcs)	Number of sealed brood (cm ²)	Number of feed (honey, beebread) (kg)	Number of frames with bees (pcs)	Number of sealed brood (cm ²)	Number of feed (honey, beebread) (kg)
18.05.	9.5±0.8	5490.0±277.5	6.8±0.8	8.9±0.9	5680.0±256.0	7.5±0.6
30.05.	12.7±1.0	5987.5±310.0	7.8±0.7	11.1±0.5	5482.0±302.5	8.2±0.8
11.06	14.1±0.9	5372.5±352.5	8.5±0.5	13.8±0.7	6122.5±302.5	8.9±0.7
23.06.	15.1±1.0	5795.0±277.5	9.5±0.8	14.9±0.8	6302.5±280.0	9.5±0.5
05.07.	16.0±0.0	5995.0±352.5	11.3±0.6	16.0±0.0	5435.0±305.0	10.5±0.6

The results showed that there is no negative impact of lactic acid on the development of bee colonies. During the experiment, no dead queens and bees were recorded in both groups.

In Russia, the studies of acaricidal activity of lactic acid were carried with the use of a 10% aqueous solution of the drug by spraying combs with bees in brood colonies, while the effectiveness of treatments ranged from 63.7±1.2% to 80.1±1.9% (Lugansky et al., 1987). No information on the effectiveness of lactic acid in the treatment of broodless bee colonies against the varroaosis in apiaries has been found in Russian Federation. The scientific findings covered in the materials of German and Swedish scientists demonstrate a high (94.2%-99.8%) acaricidal effectiveness of lactic acid in the treatment of broodless bee colonies and swarms (Kraus & Berg, 1994; Wallner & Fries, 2003; Rosenkranz et al., 2010). According to Colin M.E (1997), in the Mediterranean regions, treatment of bee colonies in the broodless period by spraying frames with bees, at a dose of 5-8 ml of 15% lactic acid on each side of the comb, reduced the number of Varroa mites to 80%. In order to maintain a low level of infestation of bee colonies with varroaosis, bee colonies should be treated four times a year with this drug.

According to the Ministry of Agriculture and Forestry of New Zealand (2001), double use of an aqueous solution of lactic acid in autumn in the above concentration by spraying combs with bees in a volume of 5-6 ml on each side causes the death of *Varroa* mites from 83 to 99%, which coincides with the results of our studies, where in some colonies the death rate of *Varroa* mites reached 84.8-94.1% after treatment with the drug. Our experimental data are also consistent with the results obtained in Turkey by Ahmet Onur Girişgin and Levent Aydin (2010), who treated bee colonies with 15% lactic acid solution in the autumn period by spraying frames with bees three times with an interval of 7 days in the volume of 5 ml on each side. The effectiveness of treatment was 87.7%, while the researchers did not observe a reduction in egg-laying of the queen bee and the death of adult bees, which is also confirmed by the results of our study. Thus, our experimental data correspond to the results of studies of other authors. We consider it necessary to continue study aimed at obtaining objective data on the effectiveness of the drug and its environmental safety for bees and bee products with a view to further use the lactic acid for the treatment of bees against varroaosis in small apiaries of Tyumen region and other regions of Russia.

Conclusion

The study found that under conditions of the South of Tyumen region double treatment (at 5 days intervals) of broodless bee colonies against varroaosis with 15.0% aqueous solution of lactic acid by spraying frames with bees in the volume of 5 ml on each side of the honeycomb reduces the number of *Varroa mites* by $90.0 \pm 2.1\%$ (apiary No 1) and $87.8 \pm 1.9\%$ (apiary No 2), respectively. At the same time, the maximum death of mites in colonies was observed during the first day after treatment. It has been established that the treatment of bees with an aqueous solution of lactic acid in the above concentration, dose and multiplicity does not have a negative impact on the development of bee colonies, their strength and food supply, and no deaths of bees and queens were detected during the observation period.

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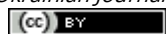
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