RESEARCH OF MECHANISMS OF THE EFFECT OF OXALIC ACID ON THE BEES AND VARROA MITES.

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Annotation

Oxalic acid is a very effective active substance against Varroa mites if applied in the absence of brood and with sufficient humidity in the hive. It does not yet cause resistance in mites, but it also has a certain effect on bees [1]. But the mechanism of its effect has not been comprehensively tested.
There is scientific evidence that:
• The most effective is the use of 3.2-3.7%% oxalic acid, diluted in sugar syrup 1:1 [2].
• After treatment with oxalic acid, the temperature in the hive increases, even in the autumn broodless period, to temperatures of +33C-42C [3].
• The use of oxalic acid can provoke the start of oviposition by the queen, despite the late autumn time.
• Oxalic acid acts on Varroa mites even without contact with them [4].

Our experiments have shown that there is a direct relationship between rising temperatures and the number and speed of mite shedding. Only where there is a rise in temperature above +30C, a significant fall of mites occur already in the first days. Acoustic monitoring of the state of bees using Apivox Smart Monitor showed that the initial rise in temperature in the hive occurs due to the active work that bees do working by their wings. Under different conditions, vibro-acoustic signals were recorded at a frequency of 125 Hz, with the 1st and 2nd harmonics, and vibro-acoustic signals at a main frequency of 250 Hz with secondary peaks at frequencies of 125 Hz and 375 Hz.
This shows that mite shedding is the result of a complex of actions.
• Death from the direct effect of the acid itself and its vapors on Varroa mites.
• Fall of the mites due to the high temperature to which bees heat up when working with their wings.
• Fall of the mites due to mechanical vibration created by bees at high speed of flapping their wings
• Death of the mites due to the fact that oxalic acid has an indirect effect on mites through the hemolymph and fat body of the bee, which partially metabolizes oxalic acid, and which in this way enters the body of mites [6].
All together creates a set of conditions for the death and subsequent fall of Varroa mites when bees are treated with oxalic acid.

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There are several ways to treat bees with oxalic acid - spraying with an aqueous solution of acid, drip treatment with a solution of acid in glycerin, drip treatment with a solution of acid in sugar syrup and sublimation. The best overall efficiency and safety indicators are for a 3% solution of oxalic acid in sugar syrup 1:1. But what happens during the processing? What is the cause of mites fall? It is believed that oxalic acid produces a contact effect. At the same time, the effect of sugar-free solutions and sublimation is less effective and lasts for less time. It is possible that sugar and glycerin adsorb moisture from the air, and this is the reason for their effectiveness. The aqueous solution directly supplies some water, which, however, quickly evaporates, after which the acid can only act if the hive is sufficiently humid. And this has been confirmed experimentally [1].
On the other hand, there is scientific evidence that the death of mites also occurs in the closed volume of a laboratory capsule in which mites and drops of corresponding liquid containing oxalic acid are placed [4]. The same trend is observed. The best effect is obtained from a solution of oxalic acid in glycerin and then in sugar syrup. Thus, it can be argued that oxalic acid produces not only a contact effect on Varroa mites, but also a general poisoning effect.
But this is not the only way oxalic acid enters the body of mites. This is confirmed by scientific data [5]. A preparation of oxalic acid, labeled with radionuclides, was introduced into the bee colony using the usual method - that is, drip. As a result, after some time, radionuclides from a solution of oxalic acid were discovered in the body of bees and their hemolymph. Radionuclides were detected in 8-9 day old brood. Radioactive markers were also found in the wax of freshly built honeycombs and in fresh honey. At the same time, the maximum infestation of bees was during the first 4 days after using the drug. According to scientists, this may mean that the results of the experiment are consistent with the hypothesis of the metabolism of oxalic acid carried out by bees. Thus, oxalic acid can act on mites in the same way through feeding on adult bees and even brood, leading to their death.

But what happens to the bees in the hive after they are treated with oxalic acid? We conducted an experiment with four families of different strengths. For the experiment, we used four families of third-generation Buckfast bees. Two families were quite strong, each occupying a full hive of Dadan - No. 4 and No. 5, one occupied about 2 frames - No. 6, and the other 5-6 frames - No. 3. The volume of the hives of all four colonies was not reduced. (Figure 1).

Figure 1 - hives of the families participating in the experiment

All families were left without any treatment throughout the entire spring-summer season of 2023. Since April, mite control was carried out in them, which by September 19 amounted to the following values: No. 3 - 2.5%, No. 4 - 19%, No. 5 - 14.4%, No. 6 - 14.5%. The remaining sealed brood available on September 19 was removed. For processing, dehydrated oxalic acid was used, which was diluted to a concentration of 3% in sugar syrup with a concentration of 1:1. The treatment was carried out dropwise, at the rate of 5-6 milliliters of the composition for each frame occupied by bees. To monitor the effectiveness of the treatment, observation of mites falling onto a sticky board was used. After treatment, the temperature in the hives was measured using digital thermometers with a remote sensor placed between the frames occupied by bees, in the center of the hive at a height of approximately the middle of the frame. Acoustic monitoring was carried out using the Apivox Smart Monitor device (apivox-smart-monitor.weebly.com).

The first treatment was carried out with oxalic acid, diluted to a concentration of 2.5% in sugar syrup with a 1:1 consistency. After 4 hours, hardware control began, which gave the following results (Figure 2). As a result, in hives No. 4 and No. 5 powerful signals were recorded in the range of 240-260 Hz, significantly exceeding all other sounds in the hive, which was indicated by the red color of the graph in the upper half of the screen. Moreover, it was in these hives that a significant rise in temperature was observed to +33.3C in hive No. 4 and to +34.3C in hive No. 5. In hive No. 6 (about 2 frames of bees), no significant sound signal was observed in the specified range, and the observed temperature was +30.2C. In hive No. 3 (about 5 frames of bees), the signal in the specified audio range was also weak. At the same time, the temperature between the frames with sensor did not exceed +24.5C.

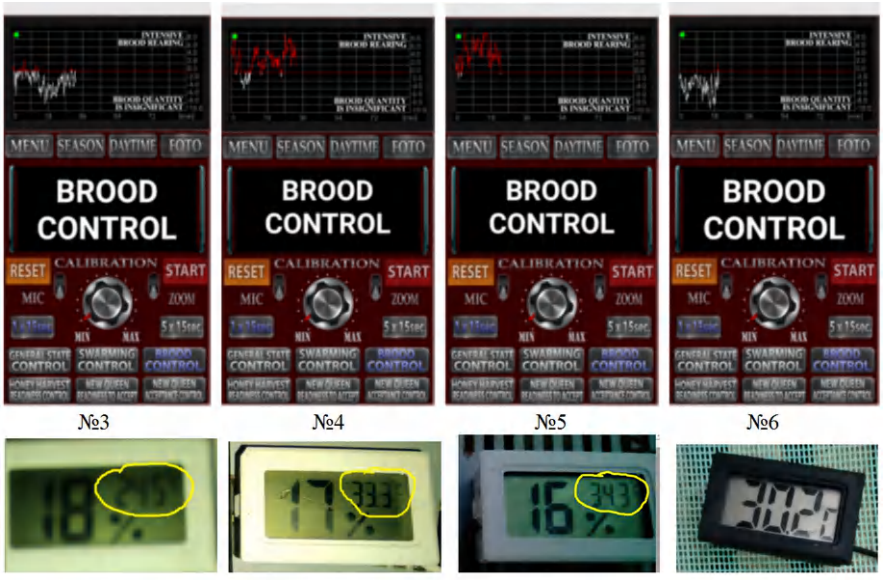

Figure 2 - results of acoustic and temperature control

Five days after treating the colonies with oxalic acid, all hives were inspected. Firstly, due to the presence of a signal in the frequency range 240-260 Hz, which corresponds to signals associated, in our opinion, with the care on open brood (aeration, heating), we monitored the frames between which sensors were installed in hives No. 4 and No.

5. It turned out that in both hives the sensors were located between the frames with honey, and there was no brood. This means that the sound signals only coincided in range with the signals of caring for open brood, but the task and reason for the work performed by the bees was completely different. The sticky boards were also checked for the presence of mites that died after treatment. The result coincided with the distribution of temperatures and sound signals recorded on the day of treatment. In hives No. 4 and No. 5, the shedding was quite significant, and according to estimates, it was 600-800 mites in each hive. About 10-15 mites fell in hive No. 3 and 2-3 mites fell in hive No. 6. On the same day, colonies in hives No. 3 and No. 6 in which practically no mites fall was observed were re-treated. We once again recorded the reaction of bees to oxalic acid, diluted in sugar syrup, and introduced dropwise into the colonies, using Apivox Smart Monitor acoustic control device (Figure 3).

Figure 3 is the result of acoustic control of experimental colonies

The figure shows that the family in hive No. 3 responded to the treatment with the appearance of a sound signal in the range of 240-260 Hz at 17-52, and by 19-21 this signal became predominant in the hive. Hives No. 4 and No. 5 were not treated again, but by the evening they also had sound signals in the specified frequency range, which most likely indicates the continued effect of oxalic acid on bees. Hive No. 6 again did not respond to repeated treatment with the appearance of a specific sound signal. After another six days, another personal control of all hives that were treated with oxalic acid was carried out. During the control, the maximum fall of mites was again recorded in hives No. 4 and No. 5. This time quite a lot of mites fell in hive No. 6. Acoustic monitoring showed that the process of exposure to oxalic acid on bees continued. In addition, the presence of nest heating signals could indicate preparation for brood rearing. After another three days, repeated control of mites drop on the sticky board was carried out. An examination showed that in hive No. 3 fell only a few mites; in hive No. 4 and No. 5 it was quite large, but we are talking about several dozen. The next control was carried out after another 10 days. Monitoring the mites drop on the sticky board showed that the fall continues at approximately the same rate as before. An examination of the families showed the presence of brood in the weakest families No. 3 and No. 6. This brood was completely unexpected, since all the frames with the sealed brood had previously been destroyed. This may mean that this is a new brood that appeared in the period after the first treatment of colonies with oxalic acid around September 20, in the presence of positive outside temperatures.

It should be added that we observed a similar reaction in families in another apiary in mid-November at positive external temperatures (Figure 4).

Figure 4 - brood in mid-November after treatment with oxalic acid

The next control of families was carried out after another 10 days. The mites drop was approximately the same as the previous time - several dozen mites in hives No. 4 and No. 5, and a single mites in hives No. 3 and No. 6. In hives No. 4 and No. 5, repeated treatment was carried out with 3% oxalic acid with sugar syrup. After 16 days, the mites drop was amounted to about 1000 mites in E4, about 600-800 in E5. This roughly corresponds to the difference in the percentage of mites infestation of these families. On average, during a month of experiments, from 57 to 75% of mites fell off. But this is very approximate.

(VERY IMPORTANT: In real beekeeping practice, repeated treatments cannot be carried out due to the subsequent significant loss of bees! Colonies, after repeated treatment, are greatly weakened until their complete death (Figure 5).

Figure 5 - bees that died after repeated treatment of the colony with oxalic acid

As a rule, treatments of the same bees are not carried out. Overwintering colonies of bees are processed in winter or late autumn at temperatures from -5C to +5C, in the absence of brood and the streets densely filled with bees. Layers and swarms are processed in spring; in summer, colonies are processed with queens blocked in the isolator and the brood completely released. But, in any of these cases, bees are always processed that have never been processed before in their lives.)

Acoustic control of these families showed that family No. 4 again responded very actively to the treatment, generating sound signals in the range of 240-260 Hz. (Figure 6).

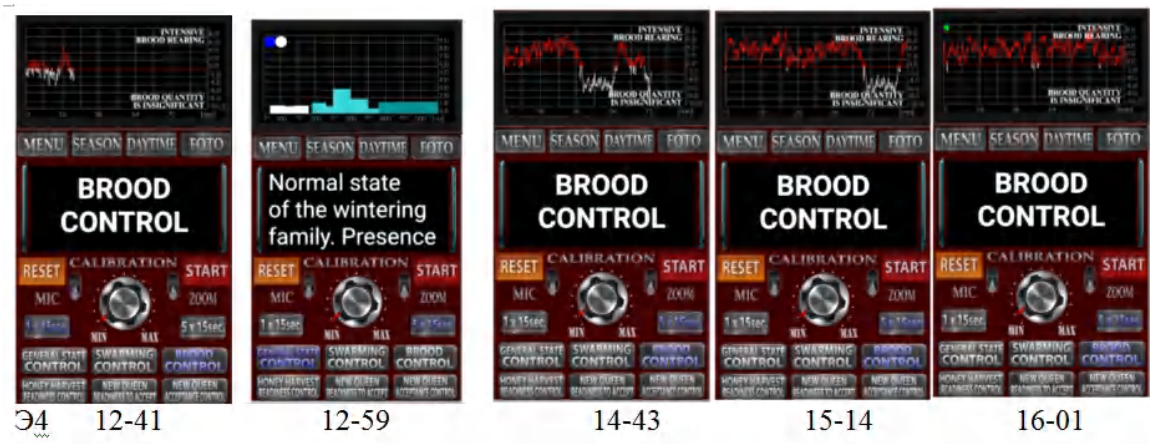

Figure 6 – results of acoustic control of families No. 4 and No. 5

At the same time, it was noted during observation in the Monitoring mode, that the signals in the region of 240-260 Hz. had a characteristic appearance, indicating that the frequency in the range of 240-260Hz. was the main frequency of the signal emitted by the bees (Figure 7).

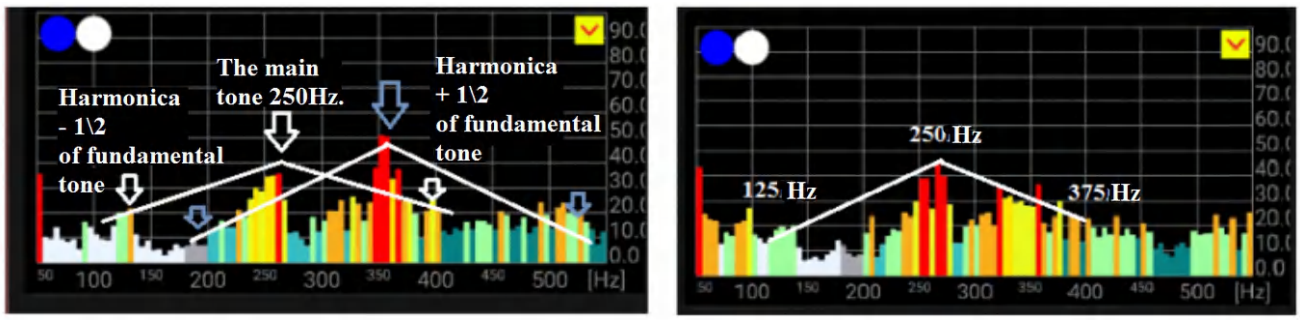

Figure 7 - spectrum of bees sounds during acoustic control of colony No. 4

The results of the experiment confirmed that the method of treatment with oxalic acid diluted in sugar syrup turned out to be quite effective and efficient. Inspection of sticky boards in experimental hives confirmed that maximum mites drop occurs in the first 5-6 days. This is consistent with the results of scientific studies, which say that it is during this time fall up to 90% of the total number of mites that will die as a result of treatment. (Figure 8)

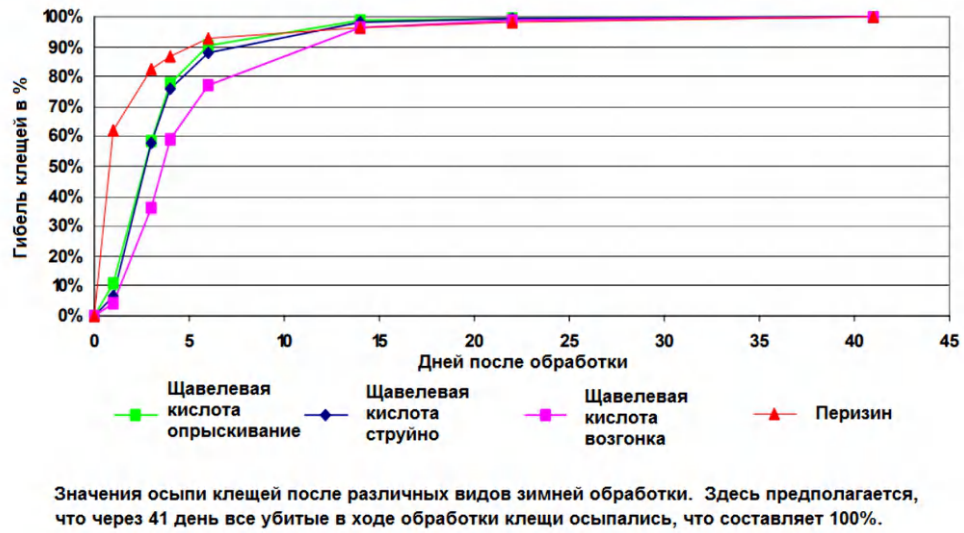

Figure 8 - graph of mites fall after treatment with oxalic acid in different variants. From work [1].

It is true that with such a treatment the temperature in the hives actually rises quite strongly, up to +35-42C (Figure 9). In our case, in strong families, we recorded temperatures up to +34.3C.

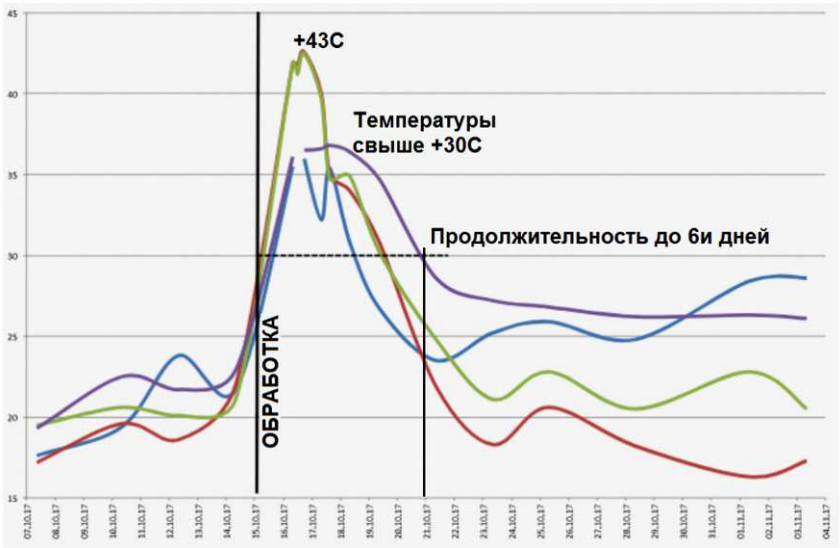

Figure 9 - graph of temperature changes in hives of families treated with oxalic acid. From work [3].

In addition, in this experiment, a completely new and previously unknown fact was fixated - the reaction of bee families to treatment with oxalic acid, in the form of the appearance of a certain characteristic sound and at the same time a fairly strong rise in temperature. In full-fledged families which occupied a full hive, was fixated an intermittent sound at the main frequency of 240-260 Hz., which was found out thanks to specific spectrum diagram (Figure 10).

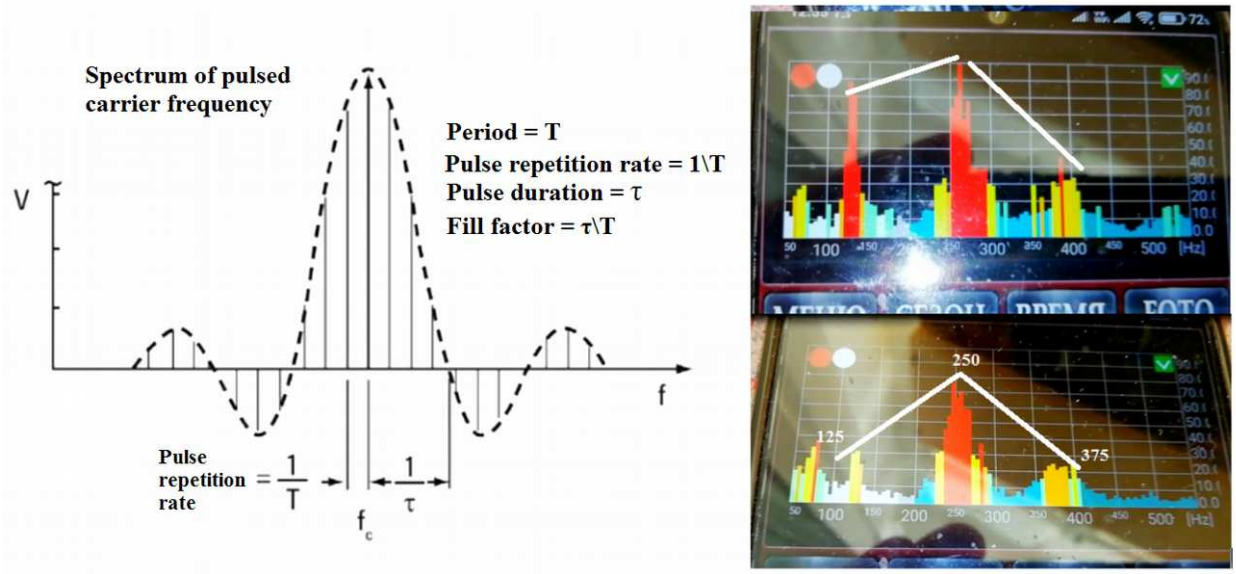

Figure 10 - graph of the spectrum of a pulse sequence filled with a carrier frequency and the spectrum of acoustic signals in full-fledged colonies of bees of the apiary, after treatment with oxalic acid.

The same sounds with the same spectrum figure are observed before swarming and when the queen is lost. We consider these sounds to be signs of "open brood care." This sound is emitted by the bees on combs with brood, periodically opening their wings and making sounds with them for a fairly short interval of time. These sounds are repeated by different bees at irregular intervals (Figure 11). As a result, an intermittent signal with a specific spectrum pattern appears.

Figure 11 - The bee on comb with brood actively flapping her wings

We believe that a possible reason for the appearance of such a signal when bees are treated with oxalic acid is the possible similarity of the smell of oxalic acid and the smell of open brood. It is known from scientific works that open brood secretes several types of acids [6]. In particular, the larvae of worker bees secrete acids - isobutanoic, isovaleric, nonanoic, fumaric, benzoic, phenylacetic, myristic, suberic and azelaic. This could explain the reason why bees perform actions similar to aerating and warming of open brood. In this case, the bees, starting to work with their wings, raise temperature so much that conditions favorable for the laying of eggs by the queen are created. It is this effect that leads to what beekeepers who use oxalic acid for autumn treatments talk about - to the beginning of egg laying by queens at the most inappropriate autumn and winter time! A completely different situation develops in

weak colonies that are in a full hive and are not separated from the main volume of the hive by an insulating partition. Such colonies, as we saw in our experiment, are not able to raise the temperature in all hive. But they can make this between two frames. We saw that the smallest families were the first to rear brood after treatment. At the same time, we also observed that the effect of acid on Varroa mites was significantly weaker in such families. The mites drop was also significantly smaller.

An additional factor in the effect of oxalic acid on mites could be its metabolization by bees. It is possible that part of the sweet liquid with acid falls on the bees during processing and, voluntarily or involuntarily, enters their body when they are cleansed of it, because according to the instructions, you need to drip syrup with acid specifically on the bees between the frames. Once on the bees and, possibly, in their food tract, oxalic acid enters their hemolymph and fat body, which the mites feed on. Thus, the acid not only has a contact effect on Varroa mites, but also directly enters their body, weakening or killing them. And such a factor may be the reason for the long fall of mites after exposure to oxalic acid. After all, as we showed earlier, mite fall can occur quite actively up to three weeks after treatment.

At the same time, the hard work performed by the bees with their wings is sometimes sufficient to raise the temperature in the hive to +40-43C. It can be assumed that the body temperature of the bees themselves can reach higher values. At the same time, it is well known that at a temperature of +38-40C, female Varroa mites do not lay eggs, and temperatures from +40C to +45°C with an hour’s exposure practically make Varroa females incapable of laying eggs. This may well be an additional factor in the effectiveness of treatment with oxalic acid. Some mites fall off under the influence of temperature, vibration and contact with acid. Some mites receive an additional dose of acid through feeding on the bees, and gradually they will die and fall off, although not so quickly. And, perhaps, there will be another group of mites in the family that did not fall on the sticky board, but survived and remained to spend winter on the bees, but at the same time lost the opportunity for spring reproduction. This may be one of the most important factors in the effect of oxalic acid on Varroa mites.

Thus, we can say that the result of treating bees with oxalic acid is a combination of factors that have a detrimental effect on Varroa mites. Namely:

• Contact effect of oxalic acid on bees and mites.
• Vibration from the active work of bees with their wings as a result of exposure to oxalic acid, leading to better shedding of mites.
• A sharp rise in the bees' body temperature, leading to the activation of mites, their movement throughout the bee's body, and better abscission.
• A sharp rise in temperature to values above +40C, leading to partial sterilization of female mites, which do not fall off after treatment, but lose their ability to reproduce in spring.
• Additional effects of acid on mites through the hemolymph and fat body of bees.
• General toxic effect on mites through breathing acid vapors. (Including the same effect, although to a lesser extent, on bees.)

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