Ukrainian Journal of Ecology

*Ukrainian Journal of Ecology*, 2018, 8(4), 143-147

*ORIGINAL ARTICLE*

# Effectiveness of oxalic acid in varroatosis in the apiaries of Tyumen Region, Russia

**A.N. Domatsky, T.F. Domatskaya**

*All-Russian Scientific Research Institute of Veterinary Entomology and Arachnology - a Branch of the Federal State Institution Federal Research Center Tyumen Scientific Center of Siberian Branch of the Russian Academy of Sciences, Tyumen, Russia.*

*E-mail: varroa54@mail.ru*

***Received: 13.11.2018. Accepted: 26.11.2018***

The mite *Varroa destructor* (Anderson and Trueman, 2000) is an obligate parasite of the honey bee *Apis mellifera* L. The pathogenic agent of the disease lives in a bee colony all year round, feeding on the hemolymph of larvae, pupae and adult insects, causing weakening and death of bee colonies, if therapeutic measures are not carried out. Ectoparasite is a vector of many pathogens of bacterial, viral and fungal infections, by giving rise to mixed diseases of bee colonies. Medicines used for treatment of bees in varroatosis are quite effective and allow to reduce the number of ectoparasite within their colonies. It was, however, pointed out that the Varroa mite has been developing resistance to most acaricides comprising amitraz, coumaphos, fluvalinate, flumethrin as active substances, which also accumulate in bee products, therefore, in many countries of the world, formic and oxalic acids are studied and proposed to control varroatosis. A study was made of the effectiveness of oxalic acid against varroatosis in the late autumn period on 35 bee colonies from two apiaries in the South of Tyumen Region. Broodless colonies were treated one time with a water-sugar solution (1:1) containing 3.2% of the drug by trickling bees into the bee spaces in the amount of 5 ml per bee space. It has been established that as a result of treatment, the number of Varroa mites has decreased in experimental colonies by 89.1 ± 1.9% (85.6-92.3%, apiary No.1)and 87.1 ± 2.2% (85.4-89.5%, apiary No.2) respectively. The maximum death of ectoparasites in experimental colonies in both apiaries was observed during the first two days after treatment. In the control groups (apiary No.1), mite mortality during the observation period corresponded to 10.1 ± 0.9% and 10.5 ± 0.7%, respectively, in apiary No.2-11.7 ± 1.1%. For more objective data on the drug, further research is needed on the effectiveness of different concentrations of oxalic acid, their effect on the physiological state of bee colonies, bees overwintering and the residues in bee products.

**Keywords:** Honey bees; *Apis mellifera* L; varroatosis; oxalic acid; effectiveness; Tyumen Region; Russia

## Introduction

The mite *Varroa destructor* (Anderson and Trueman, 2000) is an obligate parasite of the honey bee *Apis mellifera* L. The pathogenic agent of the disease lives in a bee colony all year round, feeding on the hemolymph of larvae, pupae and adult insects, causing weakening and death of bee colonies, if therapeutic measures are not carried out. In addition, the mite is a vector of many pathogens of bacterial, viral and fungal infections, by giving rise to mixed diseases of bee colonies. Medicines used for treatment of bees in varroatosis are quite effective and allow to reduce the number of ectoparasite within their colonies. It was, however, pointed out that the Varroa mite has been developing resistance to most acaricides comprising amitraz, coumaphos, fluvalinate, flumethrin as active substances, which also accumulate in bee products (Milani, 1999; Sammataro et al., 2005; Untalan et al., 2005; Wallher, 1999), therefore, in many countries of the world, formic and oxalic acids are studied and proposed to control varroatosi (Nanetti & Stradi, 1997; Nanetti et al., 2003; Rademacher & Harz, 2006; Sammataro et al., 2008; Akyol & Yeninar, 2009; Toomemaa et al., 2010; Rashid et al., 2012; Adjlane et al., 2016; Maggi et al., 2016; Maggi et al., 2017).

In the USSR, the first tests on the use of oxalic acid in varroatosis were conducted in Zakarpattia (Ukraine). The study of the use of 2% oxalic acid in different seasons of the year by spraying honeycombs in hives indicated that the most effective method is one-two treatment of bee colonies in the late autumn period, if there is no brood in families (Gaidar, 1985). Acaricidal activity of oxalic acid in the following forms was tested in laboratory and apiary environment at the All-Russian Research Institute of Experimental Veterinary Medicine (Moscow City): feeding with sugar syrup, use of vapor pressure of an aqueous solution and crystals in natural evaporation (fumigation); use of sprays obtained by burning acaricide, use of 2% aqueous solution on bees in cages, on honeycombs, in bee spaces, trickling bees in bee spaces. It has been established that the 2% aqueous solution of oxalic acid applied by spraying the bees frame at a dose of 10-12 ml. had the highest acaricidal

activity. The effectiveness of the solution on broodless boxes at temperature 27-29 °C was 88.4 ± 5.3%. The mite-fall in 24 hours after treatment corresponded to 60.3 ± 4.9%. The majority of parasites fell within 2-3 days after the first treatment. Repeated spraying increased the therapeutic effect up to 94.8 ± 4.5%. The effectiveness of single treatment of colonies in the late autumn period at a temperature of 3-11 °C was 98.4 ± 0.6% (Ivanov & Sotnikov, 1988). The use of oxalic acid in Krasnodar Region for treatment of bees in varroatosis by spraying the frames with 2% solution of the drug showed high efficiency. In 1986, tests of oxalic acid by evaporation were carried out on the apiaries of the Krasnopolyansky experimental station. It has been established that the acaricidal efficiency of the sublimates and boiling products of oxalic acid is as good as the treatment of bees with two percent aqueous solution, and exceed them by 1.3-1.5 times. In early spring, 1.5 grams were enough to fumigate one colony, and 2-3 grams of acid - in the fall, while no deaths of bee colonies occurred. In 1990, in the USSR, oxalic acid was officially registered for the treatment of bee colonies in varroatosis in a 2% aqueous solution applied by spraying bees at the rate of 10-12 ml per frame once in the spring after the flight time and twice after the end of the honey flow at intervals of 10 days at a temperature of 16 °C, as well as evaporation of a drug in a special device at the rate of 2 g per 12 frame hive twice in spring and autumn at intervals of 7-12 days at a temperature of 14 °C (Popov, 1990). Introduction of acaricides based on fluvalinate, flumetrin and amitraz on the Russian market reduced studies on oxalic acid, which led to the lack of objective data on the effectiveness of the drug in different climatic and natural conditions of Russia, in particular, the Urals and Western Siberia. Therefore, the aim of our research was to study the acaricidal activity of oxalic acid in varroatosis in Tyumen Region.

## Materials and methods

Studies were conducted in the South of Tyumen Region. The climate in the area of apiaries is characterized as sharply continental. During the year, the temperatures can fluctuate significantly. Winter is cold, but the temperature can change during the day up to 30 °C. Summer is warm, sometimes it is very hot, but in early June there may be a cold snap, sometimes even frost, spring is short. It often rains in autumn, but there are years when it is warm and dry. Annual rainfall is 360 mm. Snow cover is stable in mid-November and reaches its maximum height in late February-early March. Snow cover lasts for 5 months or more. Prevailing wind direction is west and south-west. The period of positive temperatures is 115-130 days. Average monthly temperatures for all years of monitoring are as follows: January -18.6 °C; February -17.2 °C; March -10.3 °C; April -0.2 °C; May +10.2 °C; June +15.8 °C; July +18.3 °C; August +15.8 °C; September +9.5 °C; October -0.8 °C; November -8.7 °C; December -5.5 °C.

To study the acaricidal effectiveness of oxalic acid in varroatosis in autumn, we made 2 experiments on broodless bee colonies. Bees were kept in single-hull 12-frame hives with removable bottom board. In the first experiment (apiary No.1 is in Tyumen Region), bee colonies were divided into 3 groups (experimental -10 colonies and 2 control groups of 5 colonies each). Colonies strength was 10-12 frames. For treatment of bee colonies from the experimental group, the working solution was prepared as follows: 1 kg of sugar was mixed with 1 liter of water until completely dissolved, then 75 g of oxalic acid dihydrate were added (Topolska, 2004). Bees were treated one time with the resulting solution containing 3.2% of acid by trickling into bee spaces in a volume of 5 milliliters per bee space. Colonies of the first control group were treated similarly with sugar syrup without the drug, the second control group was not treated. Dead mites were registered on a daily basis in all groups within 5 days after 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). To do this, 1 ml of the concentrate was mixed with 2 liters of water, colonies were treated with the resulting water emulsion by dripping bees into the bee spaces in the amount of 10 ml per bee space twice with an interval of 24 hours according to the instructions for use. The average daily temperature was 13.9 °C. Studies were undertaken during the last 10 days of September. The second experiment was made according to a similar scheme on the apiary No. 2, located in Zavodoukovsky District, during the first 10 days of October (apiary is located South at a distance of 97 km). The average daily temperature was 9.3 °C. Bee colonies (n=10) of the experimental group were treated with acid solution, as well as in the first experiment, control colonies (n=5) were not treated (Figure 1).

**Figure 1.** Treatment of bee colonies with 3.2% solution of oxalic acid by trickling.

Dead mites were registered in the same way. The effectiveness of treatment was determined according to the formula:

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

The data obtained were analysed using the ANOVA statistical software.

## Results and discussion

Studies have shown that the effectiveness of a single treatment of broodless bee colonies with 3.2% oxalic acid by trickling the bees into the bee spaces in the amount of 5 ml per bee space was 89.1 ± 1.9% (85.6-92.3%, apiary No.1) and 87.1 ± 2.2% (85.4-89.5%, apiary No.2) respectively. Thus, the maximum death of mites in colonies on both apiaries was observed during the first two days after treatment. In the control groups (apiary No.1), mite mortality during the monitoring period corresponded to 10.1 ± 0.9% and 10.5 ± 0.7% respectively, on apiary No.2- 11.7 ± 1.1%. The results of the studies are shown in Tables 1 and 2.

**Table 1.** Effectiveness of treatment with oxalic acid of bee colonies of apiary No.1.

| Groups, bee colonies No. | Number of dead mites after treatment by days of accounting | | | | | Number of dead mites after treatment (total) | Number of dead mites after treatment with Bipin | Effectiveness of treatment with oxalic acid (%) | Average effectiveness of treatment M ± m (%) |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | | | | |
| **Experimental (oxalic acid)** | | | | | | | | | |
| 1 | 264 | 157 | 70 | 29 | 15 | 535 | 56 | 90.5 | 89.1 ± 1.9 |
| 2 | 211 | 160 | 59 | 31 | 9 | 470 | 52 | 90 | |
| 3 | 299 | 178 | 45 | 18 | 12 | 552 | 66 | 89.3 | |
| 4 | 312 | 117 | 25 | 14 | 16 | 484 | 71 | 87.2 | |
| 5 | 197 | 122 | 25 | 17 | 12 | 373 | 48 | 88.6 | |
| 6 | 227 | 154 | 12 | 15 | 18 | 426 | 57 | 88.1 | |
| 7 | 198 | 121 | 18 | 23 | 14 | 374 | 39 | 90.5 | |
| 8 | 341 | 92 | 37 | 24 | 22 | 516 | 67 | 88.5 | |
| 9 | 199 | 98 | 25 | 31 | 24 | 377 | 63 | 85.6 | |
| 10 | 202 | 101 | 31 | 24 | 18 | 376 | 31 | 92.3 | |
| **Control (sugar syrup)** | | | | | | | | | |
| 11 | 10 | 7 | 8 | 17 | 12 | 54 | 423 | 11.3 | 10.1 ± 0.9 |
| 12 | 9 | 11 | 12 | 9 | 11 | 52 | 513 | 9.2 | |
| 13 | 12 | 11 | 18 | 9 | 9 | 59 | 522 | 10.1 | |
| 14 | 19 | 12 | 13 | 8 | 9 | 61 | 499 | 10.9 | |
| 15 | 10 | 14 | 9 | 9 | 10 | 52 | 512 | 9.2 | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Control (without treatment)** | | | | | | | | | |
| 16 | 12 | 14 | 15 | 13 | 12 | 66 | 487 | 11.9 | 10.5 ± 0.7 |
| 17 | 12 | 13 | 10 | 10 | 12 | 57 | 503 | 10.2 | |
| 18 | 15 | 11 | 10 | 11 | 12 | 59 | 563 | 9.5 | |
| 19 | 13 | 17 | 12 | 11 | 7 | 60 | 501 | 10.7 | |
| 20 | 10 | 11 | 14 | 10 | 13 | 58 | 513 | 10.2 | |

**Table 2.** Effectiveness of treating bee colonies with oxalic acid of the apiary No.2.

| **Groups, bee colonies No.** | **Number of dead mites after treatment by days of accounting** | | | | | **Number of dead mites after treatment (total)** | **Number of dead mites after treatment with Bipin** | **Effectiveness of treatment with oxalic acid (%)** | **Average effectiveness of treatment M ± m(%)** |
|---|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | | | | |
| **Experimental (oxalic acid)** | | | | | | | | | |
| 1 | 201 | 95 | 51 | 31 | 40 | 418 | 49 | 89.5 | 87.1 ± 2.2 |
| 2 | 193 | 87 | 44 | 40 | 18 | 382 | 57 | 87 | |
| 3 | 321 | 114 | 56 | 41 | 35 | 567 | 86 | 86.8 | |
| 4 | 294 | 86 | 42 | 30 | 34 | 486 | 93 | 83.9 | |
| 5 | 305 | 96 | 59 | 23 | 25 | 508 | 64 | 88.8 | |
| 6 | 212 | 56 | 49 | 38 | 26 | 381 | 75 | 83.6 | |
| 7 | 256 | 67 | 60 | 52 | 39 | 474 | 59 | 88.9 | |
| 8 | 297 | 104 | 52 | 38 | 21 | 512 | 71 | 87.8 | |
| 9 | 305 | 92 | 61 | 41 | 30 | 529 | 62 | 89.5 | |
| 10 | 187 | 103 | 91 | 50 | 39 | 470 | 80 | 85.4 | |
| **Control (without treatment)** | | | | | | | | | |
| 16 | 17 | 15 | 14 | 12 | 14 | 72 | 512 | 12.3 | 11.7 ± 1.1 |
| 17 | 18 | 16 | 14 | 10 | 15 | 73 | 514 | 12.4 | |
| 18 | 13 | 18 | 17 | 11 | 13 | 72 | 490 | 12.8 | |
| 19 | 12 | 13 | 16 | 14 | 11 | 66 | 542 | 10.8 | |
| 20 | 18 | 12 | 11 | 14 | 10 | 65 | 569 | 10.2 | |

After treatment no dead queens was recorded when controlling bee colonies of the control and experimental groups. The results of our studies are consistent with the data obtained in Tyumen Region in a single treatment of six broodless bee colonies in the late autumn period at a temperature of 7 °C by trickling bees with a water-sugar solution containing 3.2% of acid, at a dose of 5 ml per bee space. The treatment effectiveness was 84.7 ± 4.3% (Schnider, 2008). The analysis of literature data shows that in Russia, studies of acaricidal activity of oxalic acid were carried out in using the drug by spraying combs (Ivanov & Sotnikov, 1988; Burdashkina, 2004; Maslennikova & Rudenko, 2014). Currently, the company ZAO (CJSC) "Agrobioprom" (Russia) offers acaricide bisanar, an emulsion concentrate containing oxalic acid, thymol, coriander and fir oil. (Bespalova, 2014). The bisanar applied by fumigation has shown high effectiveness (Gaidar, 2014). No reliable data on the acaricidal activity of the drug applied by dripping bees into the bee spaces has not been found in the available literature. Studies in European countries (Finland, Germany, Italy, Norway, Sweden and Switzerland) and Canada to identify the effectiveness of different concentrations of oxalic acid (2.1%, 3.2% and 4.2%) have shown that sugar solutions containing 4.2% of the drug are the most effective: when administering in accordance with the protocol, the average mortality of Varroa mites is from 90.3% to 97.8%. However, in a number of tests, the 3.2% acid solution showed similar results (Nanetti et al., 2003; Rademacher & Harz, 2006), which also coincides with our results. Based on the analysis of the study results of the oxalic acid effectiveness for the treatment of bee colonies in Europe and Canada, in 2015, the Environmental Protection Agency (EPA) registered oxalic acid for use against Varroa mites in the United States. Research other countries confirms the high acaricidal activity in varroatosis and environmental safety of oxalic acid (Hatijna & Haristos, 2005; Rashid et al., 2012; Gregorc & Planinc, 2012). The data obtained from the tests thus correspond to the results of studies of other authors.

## Conclusion

Our preliminary studies revealed that in environment of the South of Tyumen Region, the effectiveness of a single treatment of broodless bee colonies in varroatosis in the late autumn period with a 3.2% water-sugar solution (1:1) of oxalic acid, applied by trickling bees into the bee spaces in the amount of 5 ml per bee space, was 87.1 ± 2.2-89.1 ± 1.9%. For more objective data on the drug, further research is needed on the effectiveness of different concentrations of oxalic acid, their effect on the physiological state of bee colonies, bees overwintering and the residues in bee products.

## Acknowledgements

The article was prepared with the financial support of the Federal Agency for Scientific Organizations of Russia within the framework of the topics of FSR No. 0371-2018-0042 "Development of new and improvement of existing methods of treatment and prevention of especially dangerous diseases of bees" and the Basic Research Program of the Russian Academy of Sciences (AAAA-A18 -118020690241-0).

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***Citation:*** Domatsky, A.N., Domatskaya, T.F. (2018). Effectiveness of oxalic acid in varroatosis in the apiaries of Tyumen Region, Russia. Ukrainian Journal of Ecology, 8(4), 143-147.