

The effectiveness of Apiguard against varroaosis (the case of Tyumen region apiaries, Russia)

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A study of Apiguard effectiveness against varroaosis was carried out in 30 colonies of bees with brood in August-September 2020 and 2021 in an apiary located in the south of the Tyumen region. Bee colonies from experimental groups (20 bee colonies) were treated twice with an interval of 14 days with a product, which is a slowly evaporating gel, packaged in 50 g aluminum containers, containing 25% thymol as an active agent. To treat bees, the container with the product was placed on the upper bars of the frames under a canvas with a preliminary installed wooden bar on it to facilitate access of the bees to the acaricide. Control colonies (10 bee colonies) were not treated. The dead mites were counted every 2 days in all groups for 30 days, for which sheets of laminated cardboard were placed at the bottom of the hives, which were removed and the number of fallen ectoparasites was counted. After the end of the experiments, during examination, frames with the remaining sealed brood were removed from the bee colonies of both groups, after which the bees were treated twice with bipin (12.5% amitraz emulsion concentrate). During the period of the experiments, the average monthly day and night temperature in August and September 2020 was 18.16°C and 12.26°C, in 2021-19.79 and 17.94°C, respectively. The effectiveness of double treatment of bee colonies with Apiguard was found with a double treatment interval of 14 days at $53.6 \pm 2.9\%$ (47.9-56.5%, 2020) and $57.8 \pm 3.2\%$ (52.7-62.1%, 2020). At the same time, the maximum mite death rate was observed after the first acaricide treatment in both cases. In the control groups (2020), the natural mortality of mites during the observation period corresponded to $13.4 \pm 1.4\%$ and $13.0 \pm 0.8\%$ (2021). During the experiments, the death of bees and queens was not observed in all groups. To obtain more objective data on the effectiveness of Apiguard, we consider it necessary to conduct similar studies in the spring-summer period before the main honey flow, taking into account the effect of the product on the development of bee colonies, the egg-laying of queens, and the state of the brood.

Keywords: Honey bees (*Apis mellifera L.*), Varroaosis, Apiguard, Efficiency, Tyumen region, Russia.

Introduction

The *Varroa destructor* mite, the causative agent of varroaosis, is a serious problem for beekeeping around the world. Despite the developed and rather effective methods of treating bees, ectoparasite continues to spread throughout the world (Nol et al., 2020). Existing technologies for managing the *V. destructor* mite include, mainly, the use of synthetic specific acaricides based on pyrethroids (fluvalinate, flumethrin), formamidines (amitraz) and organophosphates (coumaphos), organic acids (lactic, formic, oxalic), biotechnological methods (removal male bee brood, the use of trap frames, mesh stretchers, the formation of brood-free nucleus, etc.), physical methods (heat treatment of bee colonies), (Rosenkranz et al., 2010; Roth et al., 2020). However, it was noted that the varroa mite develops resistance to most of the above chemicals, the residues of which also accumulate in beekeeping products (Wallher, 1999; Sammataro et al., 2005), which is why a number of countries use veterinary thymol-based drugs to treat bees-(Apilife VAR, Thymovar, Apiguar (Mattila and Otis, 2000; Baggio et al., 2004; Emsen and Dodologlu, 2015; Gajger et al., 2020). Thymol (Thymus-thyme)-2-isopropyl-5-methylphenol, monoterpene phenol, hydroxy derivative of cymene, isomeric to carvacrol. The product has a form of colorless crystals with a specific odor and pungent taste, soluble in organic solvents, practically insoluble in water. Thymol is contained in some essential oils, for example, thyme (extracted from the leaves and seeds of *Thymus vulgaris* (common thyme) in an amount of 20-50%, from which it can be purified by rectification. Thymol is used in medicine and veterinary medicine, as well as in beekeeping against parasitic mites. In the USSR, the study of the effectiveness of thymol powder in varroaosis of bees was carried out in 1977-1979. The researchers applied the product by spraying on the upper bars of the frames on the back wall of the hive at a dose of 0.25 g per seam of bees between two combs, placed thymol in the hives in different bags, cells in an amount of 5-10 g per family. The study of the acaricidal activity of the drug was carried out in various climatic zones of the country (Crimea, Kaluga, Moscow, Omsk, Tula, and Tyumen regions). The effectiveness of treatments was found to be 73.0-97.7% (Grobov et al., 1980). The Russian Federation has now a registered medicinal product for veterinary use, Timol-V, which is a powder of white or slightly yellowish color, with a spicy-hot taste, with a characteristic odor. The instructions for the treatment of using the drug for varroaosis treatment provide for feeding the drug in 50% sugar syrup at a rate of 100 ml per 1 frame with bees, after mixing it in a syrup at the rate of 3 g per 25 l of feed. We have not found data on the effectiveness of this use of thymol in the available literature.

The thymol-containing acaricide Apiguard was tested in Voronezh, Moscow, and the Stavropol region apiaries as an alternative agent for the treatment of bee colonies with varroaosis in Russia according to the regulations for its use. The average effectiveness rate was 90.7%. There is an opinion that the effectiveness of acaricide increases when it is used in the late summer after honey flow, when the amount of bee brood decreases. Moreover, the drug can be used in spring, when the temperature is above 15 °C (Kotova, 2010; Kotova, 2012). Analysis of foreign and domestic literature on the use of thymol and preparations containing thymol for the treatment of bees with varroaosis indicates their different therapeutic efficacy, depending on the physiological state of bee colonies, ambient temperature, season, climate, housing conditions, application process (Baggio et al., 2004; Wahida et al., 2010; Gregorc et al., 2018; Gregorc and Sampson, 2019). In this regard, the purpose of our research was to study the acaricidal efficiency of Apiguard at varroaosis in apiaries of the Tyumen region.

Materials and Methods

Apiguard (Apiguard) is a slowly evaporating gel, packaged in 50 g aluminium containers, containing 25% thymol as an active agent and additional components that ensure slow evaporation of thymol and prolong the effect of the product (manufacturer Vita (Europe) limited, England). For treatment purposes, the container with the drug is placed on a honeycomb frame under a canvas, with a preliminary installation of a wooden bar to facilitate the access of bees to the acaricide.

The studies were carried out in the south of the Tyumen region in the same apiary in August-September 2020 and 2021. During the period of the experiments, the average monthly day and night temperatures in August and September 2020 were 18.16 and 12.26°C, in 2021-19.79 and 17.94°C, respectively.

For the experiments in each season, 15 bee colonies with brood were selected, which were divided into 2 groups: experimental (10 colonies) and control (5 colonies). Bee colonies were kept in twelve-frame hives with removable bottoms. The strength of the experimental and reference colonies was 12 frames. After the collection of commercial honey from the hives and clinical examination, the bee colonies of the experimental groups were treated with Apiguard acaricide twice in the way described above with an interval of 14 days; the control bees were not treated. Dead mites were counted every 2 days in all groups for 30 days: sheets of laminated cardboard were placed at the bottom of the hives, which were removed and the number of fallen ectoparasites was counted. After the end of the experiments, during examination, frames with the remaining sealed brood were removed from the bee colonies of both groups, after which the bees were treated twice with bipin (12.5% amitraz emulsion concentrate). To do this, 1 ml of the concentrate was mixed with 2 l of water, the families were treated with the resulting aqueous emulsion by showering bees through interframe spaces in a volume of 10 ml per bee seam between two combs twice with an interval of 24 hours according to the instructions for use of the product. The mites were counted in a similar manner. The effectiveness of the treatment was evaluated using the following formula:

Effectiveness of Apiguard (%) = $\frac{\text{the number of dead mites after treatment with Apiguard}}{\text{the number of dead mites after treatment with Apiguard and bipin}} \times 100$

The data obtained were analyzed using the ANOVA statistic program (Fig 1).



Fig. 1. Treatment of bee colonies with Apiguard.

Results and Discussion

Studies have shown that the effectiveness of double treatment of bee colonies with Apiguard with an interval of 14 days was $53.6 \pm 2.9\%$ (47.9-56.5%, 2020) and 57.8 ± 3.2 (52.7-62.1%, 2021), indicating that there are no significant differences between the indicators. At the same time, differences in the effectiveness of treatment were observed in individual colonies. The maximum death rate of mites was observed after the first acaricide treatment in both cases. In the control groups (2020), the natural mortality of

the mites during the observation period corresponded to $13.4 \pm 1.4\%$ and $13.0 \pm 0.8\%$ (2021). During the experiments, the death of bees and queens was not observed in all groups. The research results are given in Tables 1 and 2.

Table 1. Effectiveness of Apiguard treatment of bee colonies in 2020.

Groups, of colonies	No. bee	The number of dead mites		Number of dead mites after the treatment with Apiguard (Total)	The number of dead mites after Bipin treatment with Bipin (Total)	Effectiveness of treatment with Apiguard (%)	Average treatment effectiveness $M \pm m$ (%)
		First treatment	Second treatment				
Experimental (Apiguard)		10.08-23.08	24.08-06.09				
1	295		124	419	456	47.9	53.6 ± 2.9
2	301		187	488	395	55.2	
3	391		301	692	537	56.3	
4	411		205	616	551	52.8	
5	399		198	597	438	55.7	
6	454		221	675	523	56.3	
7	295		154	449	438	50.6	
8	424		285	709	624	51.2	
9	365		287	652	567	53.4	
10	385		299	684	525	56.5	
Control							
11	59		75	134	1069	12.5	13.4 ± 1.4
12	85		98	183	1333	13.7	
13	98		55	153	1151	11.6	
14	79		81	160	1147	13.9	
15	87		95	182	1198	15.2	

Table 2. Effectiveness of the treatment of bee colonies with Apiguard in 2021.

Groups, of colonies	No. bee	The number of dead mites		Number of dead mites after The Treatment with Apiguard (Total)	The number of dead mites after Bipin treatment with Bipin (Total)	Effectiveness of treatment with Apiguard (%)	Average treatment effectiveness $M \pm m$ (%)
		First treatment	Second treatment				
Experimental (Apiguard)		07.08-21.08	22.08-04.09				
1	334		313	647	581	52.7	57.8 ± 3.2
2	298		159	457	325	58.4	
3	384		281	665	481	58.0	
4	385		248	633	441	58, 9	
5	392		315	707	564	55.6	
6	295		207	502	435	53.6	
7	401		197	598	384	60.9	
8	325		294	619	392	61.2	
9	465		300	765	467	62.1	
10	344		245	589	448	56.8	
Control (without treatment)							
11	68		85	153	1299	11.8	13.0 ± 0.8
12	98		100	198	1348	14.7	
13	75		69	144	1074	13.4	

14	85	71	156	1286	12.1
15	91	82	173	1315	13.1

Thymol-based acaricides are one of the alternative treatments for varroaosis in bee colonies. Analysis of the results of studies on the use of thymol and thymol-containing drugs against the *V. destructor* mite in many countries indicates their varied therapeutic efficacy in different climatic zones. Acaricides containing thymol are recommended for use when the ambient temperature is between 15.0 and 30.0°C. Thymol evaporation also depends on the strength of bee colonies, the presence of sealed broods, and the formulation of the drug (Baggio et al., 2004; Gregorc and Sampson, 2019). The results of our studies on the effectiveness of Apiguard (53.6%-57.8%) differ from the data obtained in the Voronezh, Moscow and Stavropol regions (90.7%), where the experiments were carried out in spring at temperatures above 15°C and in summer after the removal of commercial honey from the hives (Kotova, 2010). One of the reasons for the low acaricidal activity of Apiguard in the Tyumen region, in our opinion, is the sharp changes in air temperature during the day, especially at night (August 2020: the minimum average daily temperature is +13.17°C, the maximum is +24.03°C; August 2021: the minimum average daily temperature +13.17°C, maximum -28°C. Analysis of temperature data indicates that August and September were warmer in 2021 compared to 2020. Our experimental data are consistent with the results of studies in other countries, where the effectiveness of the product in a continental climate was 42.0 and 46.0% (Gregorc, 2005; Gregorc and Planing, 2005; Gregorc et al., 2018). A similar result (47.3%) was obtained in a study of the effectiveness of acaricides in Croatia (Gajger et al., 2020).

In countries with a warm climate, the effectiveness of acaricide treatments of bees is higher. Studies conducted in Italy show that Apiguard reduced the rate of infestation of bee colonies with *V. destructor* mites by 76.1% and 82.6% (Baggio et al., 2004; Giacomelli et al., 2015). In Algeria, the use of Apiguard for the treatment of bees caused the death of ectoparasites in the amount of 81.0%, 72.6%, and 93-97% (Wahida et al., 2010; Adjlanen and Haddad, 2011; Adjlanen et al., 2016). A study of the acaricidal activity of Apiguard in Mexico revealed a high toxicity of the product against varroa mites, which was 93% and 97% for adult bees-94% and 95% for sealed brood (May-Itzá et al., 2007). Thus, the analysis of the obtained own data and the results by studies of other authors once again confirm the dependence of the treatment efficiency of Apiguard at varroaosis on the ambient temperature.

Conclusion

As a result of the studies carried out, it was found that in the conditions of the South of the Tyumen region, the efficiency of double treatment of bee colonies with bred with Apiguard in August-September 2020 and 2021 with an interval of 14 days was $53.6 \pm 2.9\%$ and $57.8 \pm 3.2\%$, respectively. The death of bees and queens during treatment and after it was not observed. To obtain more objective data on the effectiveness of the product, we consider it necessary to conduct similar studies in the spring-summer period before the main honey flow, taking into account the effect of the product on the development of bee colonies, the egg-laying of queens, and the state of the brood.

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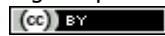
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