

ORIGINAL ARTICLE

Study of fluvalinate residues in honey and wax after treatment of bee colonies in varroatosis

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In three experiments on 18 bee colonies of the apiaries of Tyumen Region, the fluvalinate level in honey and wax after treatment of bee colonies with the following veterinary preparations has been studied: Tanis, Acaricidal bath, Varroplast M. Bee colonies were treated with drugs according to the application instructions. 7 days after treatment, wax and honey samples were taken from each colony and studied for active substance using gas-liquid chromatography. 36 wax samples and 18 honey samples were studied in total. A study does not reveal any fluvalinate residues in samples of honey and wax after treatment of bee colonies with Tanis. In bee colonies treated with Acaricidal bath, the fluvalinate level in wax selected from the brood frames averaged 0.97 ± 0.12 mg/kg, from honey frames - 0.12 ± 0.01 mg/kg in honey - 0.0026 ± 0.0002 mg/kg. A significant number of fluvalinate was found in bee colonies treated with Varroplast M. The fluvalinate level in wax samples from brood frames was 1.4 ± 0.09 mg/kg. Studies of wax taken from the honey frames indicated that there were fluvalinate residues of average 0.28 ± 0.08 mg/kg, the amount of drug in honey was 0.005 ± 0.001 mg/kg. It was found that the greatest number of fluvalinate residues is present in wax samples obtained from the brood frames. In the experiment with Acaricidal bath, the active substance level in wax from the brood combs significantly exceeded similar indicators of wax taken from honey frames ($t=7.08$). In the experiment with Varroplast M, the number of fluvalinate in wax samples taken from the brood frames is significantly higher than the data on wax from honey frames ($t=9.3$). Analysis of the results of own studies and literature data shows that the honey products should be monitored for acaricides residues, used to treat bee colonies in varroatosis in the apiaries of the Russian Federation.

Keywords: Bee colonies; varroatosis; fluvalinate; residues; honey; wax

Introduction

Varroatosis is an invasive disease of bee colonies caused by Varroa destructor mite. Ectoparasite lives in a bee colony all year round, feeding on the hemolymph of larvae, pupae and adult insects. The economic damage from the invasion is compounding because the mite is a vector of many pathogens of bacterial, viral and fungal infections. Without treatment measures, bee colonies die within two-five years (Gregorc et al., 2018). As shown by the analysis of current means for fighting against varroatosis used in Russian beekeeping, a significant part of them is represented by chemical preparations based on fluvalinate and amitraz, which poses a threat to contaminate honey products with residues of drugs, and therefore constant monitoring of the quality of honey, wax, bee glue and pollen is required. Honey products, such as honey, are widely used as food and medicines, their contamination could pose a serious risk to human health (Al-Waili et al., 2012; Bonzini et al., 2011; Wilmart et al., 2016). The fact that among acaricides used, bromopropylate, coumaphos and fluvalinate are most often found in honey and wax has been established. These active substances are fat-soluble and non-volatile compounds capable of accumulating in beeswax after treatment of bees. Ingredients migrate from wax into honey during storage (Wallner, 1999). Re-treatment contribute to increasing the number of acaricides residues in wax. It was found that the wax and honey get contaminated after the use of veterinary preparations in accordance with the Swiss regulations in autumn, after honey collection. During the following spring, the residues level found in different products after treatment with acaricide had been decreasing as follows: brood combs > honey combs > sugar feed \geq honey. (Bogdanov, 2006). Monitoring of pesticide residues in honey, wax, and bees helps assess a potential risk of these products for human health and presents data on the scale of treatment with pesticides that were used on field crops surrounding the hives, as well as on veterinary preparations used to treat bees. For the treatment of bee colonies in varroatosis, different preparative forms of acaricides, including fluvalinate, are widely used in the Russian Federation: polymer, wooden plates, cardboard thermal plates, emulsion concentrates. In view of this, steady monitoring of honey products for residues of the active substance is required. The aim of our study was to study

honey and wax for fluvalinate residues after treatment of bee colonies with Tanis, Varroplast M., Acaricidal bath. 3 experiences in the apiaries of South of Tyumen Region have been made.

Materials and methods

General situation of study area

Tanis - a veterinary preparation, is a thermal cardboard plate 20 × 50 × 1 mm in size, containing, as an active ingredient, 0.5 mg of fluvalinate. Fluvalinate - C₂₆H₂₂ClF₃N₂O₃ - an insectoacaricide of the cyano-pyrethroid class of compounds, containing in preparations used to protect plants and honey bees against pests and parasites. Acaricide has a high therapeutic efficacy in varroaosis and does not have a negative impact on development of bee colonies (Domatskaya & Domatsky, 2017). In the first experiment, 6 brood bee colonies with strength of 16 frames were selected for studies. Treatments were done three times at an interval of 4 days according to the drug application instructions. To do this, 2 smoking plates on special substrates were applied into the hives through the lower bee-entrances which were closed for 20 minutes. During the treatment period, 3.0 mg of the active substance was applied into each bee colony. 7 days after the third treatment, sealed honey and wax samples were taken from all colonies and studied for fluvalinate residues.

In the second experiment, honey and wax were studied for acaricide residues after treatment of bee colonies with Varroplast M (the drug is a wooden plate 30 × 200 × 1.2 mm in size, containing 600 mg of fluvalinate). Preliminary studies have shown that the treatment of bee colonies with Varroplast M reduces the number of *V. destructor* mites in bee colonies by 91.0 ± 100.0% (Domatskaya et al., 2017). At the end of May, 6 brood bee colonies with strength of 14 frames were selected for study, which were treated with Varroplast M by evenly placing 2 plates between the combs for a period of 30 days (Figure 1), in this case, 1200.0 mg of fluvalinate was applied into each colony. At the end of the treatment period, after 7 days, samples of sealed honey and wax were taken from all colonies and studied for residues of the active substance.

In the third experiment, 6 bee colonies were treated with Acaricidal bath in August. Acaricidal bath is a technical filter board sheet 250 × 200 × 1 mm in size, including 200 mg of fluvalinate. For the treatment of bees, 2 baths (400.0 mg) were applied into the bottom of hives through the lower bee-entrance in such a way as to make bees flying in and out constantly contact the treated surface (Figure 2) (Domatskaya & Schneider, 2009). The treatment time was 24 days. 7 days after the treatment, honey and wax samples were taken from the colonies and studies for fluvalinate residues.

In each experience, 12 wax samples and 6 honey samples were selected for study. A total of 54 studies of honey products samples have been done. Fluvalinate residues in honey and wax were determined in accordance with the "Guidelines on determining fluvalinate in honey and wax by gas-liquid chromatography" approved by the Ministry of Health of the USSR on July 29, 1991 under No. 6223-91. Metrological characteristic of the method: minimum detectable amount - 0.2 ng, lower limit of detection - 0.01 mg/kg, (Guidelines, 1995).

Statistical analysis

The results were processed using the "ANOVA" program.

Results and analysis

The studies have shown that there were no fluvalinate residues in samples of honey and wax after treatment of bee colonies with Tanis. In bee colonies treated with Acaricidal bath, the fluvalinate level in wax, selected from the brood frames, averaged 0.97 ± 0.12 mg/kg (0.81-1.13 mg/kg), from the honey frames - 0.12 ± 0.01 mg/kg (0.10-0.13 mg/kg), in honey itself - 0.0026 ± 0.0002 mg/kg (0.002-0.003 mg/kg) (Table 1).

Table 1. Fluvalinate level in wax and honey (mg/kg, M ± m).

Bee colony No.	Name of veterinary preparation	Wax from brood frames	Wax from honey frames	Honey
1	Acaricidal bath	0.91	0.13	0.002
2		0.81	0.11	0.003
3		1.12	0.11	0.002
4		0.94	0.13	0.003
5		0.93	0.12	0.003
6		1.13	0.1	0.003
Average, M ± m		0.97 ± 0.12	0.12 ± 0.01	0.0026 ± 0.0002
7	Varroplast M	1.4	0.21	0.005
8		1.3	0.3	0.006
9		1.5	0.22	0.005
10		1.4	0.41	0.006
11		1.3	0.22	0.005
12		1.3	0.33	0.005
Average, M ± m		1.4 ± 0.09	0.28 ± 0.08	0.005 ± 0.001

m	Tanis			
13		not detected	not detected	not detected
14		-	-	-
15		-	-	-
16		-	-	-
17		-	-	-
18		-	-	-

The maximum number of fluvalinate was found in bee colonies treated with Varroplast M. The fluvalinate level in wax samples from brood frames was 1.4 ± 0.09 mg/kg (1.3-1.5 mg/kg). Studies of wax taken from the honey frames indicated that there were fluvalinate residues of average 0.28 ± 0.08 mg/kg (0.21-0.33 mg/kg), presence of the drug in honey corresponded to 0.005 ± 0.001 mg/kg (0.006-0.005 mg/kg).

The analysis of obtained data shows that the maximum number of fluvalinate residues is present in wax samples obtained from the brood frames. In the experiment with Acaricidal bath, the active substance level in wax from the brood combs significantly exceeded similar indicators of wax taken from honey frames ($td=7.08$). In the experiment with Varroplast M, the number of fluvalinate in wax samples taken from the brood frames is significantly higher than the data on wax from honey frames ($td=9.3$). The low fluvalinate level in honey in both experiments was, however, noted. The content level of fluvalinate residues in honey products is affected by the drug dosage used to treat bees.



Figure 1. Bee colonies treatment against varroatozosis with Varroplast M.



Figure 2. Bees treatment with Acaricidal bath.

Discussion

The results of our studies correspond with those obtained by Tamim Alia, Abdullah Hatoum, Khawla Muhammad (Tamim et al., 2011), where after the bees were treated once with fluvalinate, the active substance level in honey was significantly less than in wax and was 0.000001 mg/kg and 0.02583 mg/kg, respectively. The study of 135 honey samples taken from bee colonies in 9 regions of Turkey for drugs residues including fluvalinate has found no residues in the product (Bilgili & Selçukoğlu, 2017). According to (Bogdanov, 2006) the study of fluvalinate residues in spring of the following year after the previous autumn treatment of bees with Apistan showed that there were residues of the active substance in wax from the brood frames in the amount of 2.9 mg/kg, in wax from honey combs – 0.1 mg/kg, in honey - \leq 0.001 mg/kg. Studies by The European Agency for the Evaluation of Medicinal Products (EMA/MRL/021-REV1/95) of 25 bee colonies treated with Apistan showed the significant fluvalinate residues in wax (from 0.2-5.5 up to 26.9 mg/kg), the amount of active substance in honey was mainly 10 μ g/kg and only in two samples - 12 and 42 μ g/kg (EMA/MRL/021-REV1/95, 1995). In Greece, 58 honey samples were analyzed. 60% of samples studied, contained less than 1 μ g/kg of active substance, while the remaining samples were found to contain concentrations up to 3.82 μ g/kg. The fluvalinate level in honey has not increase over the years. The fluvalinate level in 114 wax samples (depending on its type) studied during this period was 0.4-30.14 mg/kg (Tsigouri et al.,2003). According to (Bogdanov et al., 1999), the fluvalinate level in brood combs varied from 0.2 to 7.3 mg/kg during 15 months after treatment with an average value of 1.9 mg/kg. With constant use of fluvalinate, acaricide residues had been increasing and reached 40-60 mg/kg after 6 months. The amount of active substance in honey in the same colonies was 5 times lower than in brood combs. The fluvalinate level in honey was mostly 0.003 mg/kg. The data obtained from the tests thus correspond to the results of studies of other authors.

Conclusion

The studies revealed fluvalinate residues in bee colonies treated only with Acaricidal bath and Varroplast M. The maximum amount of the drug was found in wax samples taken from the brood frames. Lower concentrations of the active substance are registered in wax from honey frames, as well as in honey itself. Analysis of the results of own studies and literature data shows that the honey products should be monitored for acaricides residues, used to treat bee colonies in varroaosis in the apiaries of the Russian Federation.

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