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THE INFLUENCE OF FORMIC ACID, OXALIC ACID AND ESSENTIAL OILS ON THE FREE ACIDITY IN HONEY

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ABSTRACT

The aim of the study was to monitor the changes in the free acidity of honey after administration of three anti-varrotic treatments with the use of formic acid (FA), oxalic acid (OA) and essential oils as the active substances in commercial preparation. To the first experimental group, 60 % FA was administered by vaporization using the vaporizer Nassenheider Professional® (group F). OA was administered by the contact in the form of 26.8 % (w/w) glycerine solution of OA on special workshop towels (group O). Essential oils were administered in the form of the commercial product Bisanar® (group B). The control group received no treatment during the experiment. Although the acidity of the honey in the B group during the experiment did not show significant difference compared to the control group ($p = 0.769$) and only a slight difference was determined in the O group ($p = 0.416$), the F group showed significant increase of the values compared to the control group ($p = 0.015$). The average value of free acidity in honey of this experimental group did not decrease notably under the legislative limit after two weeks following removing the treatment from the hive. Free acidity in the F group reached the value of 57.50 ± 25.19 mEq/kg (milliequivalents per kg) at the end of the experiment. Treatments by OA and Bisanar® are more suitable to supress varroosis during the summer without significant increase of free acidity in honey.

Key words: Varroosis; formic acid; oxalic acid; essential oils; honey; acidity

INTRODUCTION

In beekeeping practice, beekeepers are during the summer more and more inclined to apply drugs designed to suppress varroosis. However, most synthetic treatments cannot be administered into the colony when honey designated for human consumption is present in the hive. Residues of synthetic active substances must not be present in honey, or their maximal residual limit (MRL) must not be exceeded. The manufacturers of treatments meet these criteria when the information on the product states that the treatment cannot be applied when there is honey in the hive.

For this reason, a beekeeper seeking to protect the winter generation of bees from varroosis reaches for treatment with so-called "natural active substance". There is no MRL for these substances and essential oils have the status FAO GRAS (Generally Recognised as Safe) for a value of up to 50 $mg.kg^{-1}$. This, however, does not mean that they cannot influence the sensory characteristics of honey, including its aroma and taste. Changes to the aroma and taste of honey, caused by essential oils, are well-known (Bogdanov *et al.*, 1999). This article is not aimed at the honey smell but at the honey taste, in particular the acidity of honey. The natural formic acid (FA) content in honey is in the range from 5 to 600 $mg.kg^{-1}$ (Capolong *et al.*, 1996) and the content of oxalic acid (OA) – in the range from 1 to 255 $mg.kg^{-1}$ (Kary, 1987). Free acidity is then approximately 7.1 – 7.5 mEq/kg (milliequivalents per kg) for acacia (*Robinia pseudoacacia*) and 22.2 – 26.8 mEq/kg – for

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honeydew honey. Humans were capable of sensory recognition of acidity in honey in the range of 150 – 300 mg.kg^{-1} for FA and 300 – 400 mg.kg^{-1} for OA in acacia honey. The taste threshold for acacia honey is about half of those for honeydew honey (i.e., taste threshold 300 – 600 mg.kg^{-1} for FA and 700 – 900 for OA), because the honeydew honey has a much stronger aroma and can support more acidity than the acacia honey (Bogdanov *et al.*, 1999). Therefore, evaluation of the samples by measuring the free acidity is more sensitive for assessment of acidity change compared to evaluation based on sensory criteria. Numbers are better suited to determine which honey is more acidic. The following decrees apply to the physical and chemical parameters of honey in Slovakia: Decree 41/2012 and Decree 106/2012. In Decree 41/2012, the maximum limit for free acidity of honey can be found. Specifically, it is 50 mEq/kg. If this limit is exceeded, like the case of water content of honey, the product must not be designated as honey and must not be introduced to the market.

The aim of the work was to monitor the changes in the acidity of honey after the application of formic and oxalic acid and of the drug Bisanar® containing essential oils.

MATERIAL AND METHODS

For the needs of the experiment, 12 bee colonies were selected at the Maša apiary, near Liptovský Hrádok (GPS 49.034590, 19.767316), which were divided into four groups. The F group was given the formic acid (FA) active substance, the O group – oxalic acid (OA), the B group – Bisanar® and the C group was left untreated. A sample of honey was taken from the hives before the individual drugs were put into the hive. At each collection, honey was taken from the honeycomb, at the interface between uncapped and capped honey cells. During the entire course of the experiment, the colonies were not fed. The sampling was carried out at weekly intervals and the samples were subjected to laboratory analyses immediately after sampling on the same day.

A standard Nassenheider Professional® horizontal vaporiser (Figure 1) was used for the treatment with the FA active substance, which was filled with 300 ml of 60 % FA (VWR, Belgium). After a week, during sampling, acid vapor volume was recorded (by measure

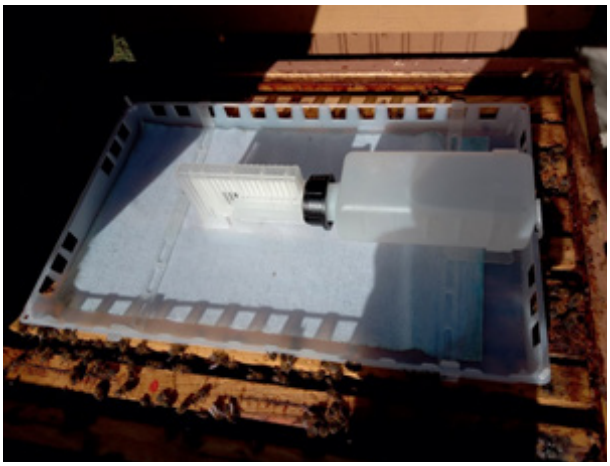

Figure 1. Experimental treatment by FA to supress varroosis (photo: M. Staroň, 2022)

of the rest and its subtracting from applied volume) and the container was refilled with 60 % FA. The calculation of the vapor was repeated another week when the vaporiser was removed (two-week exposure). The consumption per hive thus represented the maximum possible values resulting from practice and reached a variance of 510 to 600 ml during two weeks of application.

The treatment with the OA active substance was performed by special workshop towels – Universal Cleaning Cloth Roll Blue 3-Ply for Workshop Leisure (VDEMA, Germany) impregnated with a 26.8 % of OA (w/w; VWR, Belgium) glycerine solution (Figure 2). The size

Figure 2. Experimental treatment by OA to supress varroosis (photo: M. Staroň, 2022)

of the towel was chosen to fully absorb 12 ml of such a solution without leaving unnecessary dry spots on the towel. Dosing took place in a zip lock bag, so that the correct absorption of the entire exact volume into the towel could be observed. The cloth was applied to the hive by hanging it over the top bar of the frame, directly to the brood. The towels were left in the hive for the entire duration of the experiment and the bees gradually took them out of the hive throughout the experiment.

Bisanar® (AO Agrobioprom, Russia) was applied 3 times (in week intervals) in the dose and manner indicated in the package leaflet of the treatment by sprinkling on the bees (Figure 3) in the aisles between the frames at a dose of 100 ml/beehive/treatment with an interval of one week. According to the producer information, active substances in Bisanar® were thymol (100 $mg.kg^{-1}$) and essential oils of fir (*Abies* sp.) and coriander (*Coriandrum sativum*).

No reagent was administered to the control group.

Figure 3. Experimental treatment by Bisanar® to supress varroosis (photo: M. Staroň, 2022)

At the beginning of the experiment (before the first administration of the treatment) and subsequently for four weeks, honey (approximately 50 ml from each bee colony) was collected from the interface of the capped and uncapped cells of the honeycombs exactly at weekly intervals. All the treatments (FA, OA and Bisanar®) were started in selected bee colonies immediately after the first honey samplings (4^{th} August 2022). These samples were initially processed in the laboratory (free from wax residues), by filtering through gauze (Figure 4). Honey samples from each bee colony (n = 12 per collection, i.e. n = 3 for each group) were analysed separately. Subsequently, the samples were analysed in term of the water content (determined by refractometry) and free acidity (determined by titration). Free acidity was expressed in mEq/kg (milliequivalents per kg), which represents consumption of 1 $mol.dm^{-3}$ sodium hydroxide needed to neutralization of all free acids in 1 kg of honey. The honey analytic methodologies used were in accordance with IHC (2009).

Figure 4. Sample processing prior to laboratory examination (photo: M. Staroň, 2022)

STATISTICS

JASP 0.16.4.0 and SPSS 16.0 were used to obtain the results of descriptive statistics, as well as for the actual testing and mutual comparison of individual groups. The water content data were used in the testing directly. Data of the free acidity were adjusted by calculating the difference between the measured value and the initial level. To assess the normality of the data distribution, the skewness and kurtosis of the data distribution were calculated. Individual factors were evaluated by ANOVA test and groups were compared against control and each other using post-hoc analysis by non-parametric Games-Howell test. The choice of the non-parametric Games-Howell test was based on the finding that the F group showed a high left-sided skewness of the data (Skewness = 1.324; SE = 0.58), as well as the fact of the low number of

Table 1. Water content, free acidity and difference of free acidity during the experiment

Group	Parameter	Date of collection and analysis of the samples					Sig.*	SE*
		4 August 2022	11 August 2022	18 August 2022	25 August 2022	31 August 2022		
F (n = 3)	Mean water content (%)	16.87 ± 0.50	16.73 ± 0.46	17.60 ± 1.04	16.87 ± 0.61	17.13 ± 0.81	0.450	0.458
	Mean free acidity (mEq/kg)	18.17 ± 0.29	37.00 ± 17.59	71.83 ± 56.50	63.67 ± 31.15	57.50 ± 25.19	-	-
	Mean difference of free acidity (mEq/kg)	-	18.83 ± 17.82	53.67 ± 56.66	45.50 ± 31.04	39.33 ± 25.07	**0.015**	8.730
O (n = 3)	Mean water content (%)	17.40 ± 1.22	19.13 ± 2.12	19.13 ± 1.62	18.53 ± 1.53	18.20 ± 2.09	0.598	0.595
	Mean free acidity (mEq/kg)	18.33 ± 3.79	19.17 ± 2.75	21.33 ± 4.93	22.26 ± 7.29	19.50 ± 6.56	-	-
	Mean difference of free acidity (mEq/kg)	-	0.83 ± 1.04	3.00 ± 3.61	3.93 ± 3.52	1.17 ± 3.79	0.416	0.819
B (n = 3)	Mean water content (%)	17.80 ± 1.11	17.60 ± 0.72	17.87 ± 1.14	17.60 ± 0.87	17.40 ± 1.22	0.998	0.481
	Mean free acidity (mEq/kg)	16.33 ± 0.58	16.50 ± 2.65	16.33 ± 1.44	16.50 ± 3.97	15.17 ± 2.25	-	-
	Mean difference of free acidity (mEq/kg)	-	0.17 ± 3.22	0.00 ± 1.80	0.17 ± 4.19	-1.17 ± 1.76	0.769	0.689
C (n = 3)	Mean water content (%)	18.00 ± 2.40	17.33 ± 1.80	17.60 ± 1.78	17.80 ± 1.83	17.93 ± 1.67	-	-
	Mean free acidity (mEq/kg)	19.67 ± 4.25	19.83 ± 4.04	20.00 ± 2.50	20.67 ± 5.92	20.67 ± 3.75	-	-
	Mean difference of free acidity (mEq/kg)	-	0.17 ± 2.02	0.33 ± 2.08	1.00 ± 1.80	1.00 ± 0.87	-	-

F – experimental group treated by formic acid, O – experimental group treated by oxalic acid, B – experimental group treated by Bisanar®, C – control group without treatment, mEq – milliequivalent

*Games-Howell, α = 0.05, Sig. – p values form comparison of test group versus control group, SE – Standar Error for Games-Howell test – not specified

colonies in the groups. The relationship between water content and free acidity was determined by a correlation test according to the Pearson distribution.

RESULTS

All mean values of evaluated parameters during the experiment are presented in the Table 1 and the spread of water content and free acidity difference data in individual groups are shown in the Figures 5 – 6.

At the beginning of the experiment, all groups of bee colonies showed average water contents in the honey ranging from 16.87 ± 0.50 % to 18.00 ± 2.40 % (Figure 7). That is within the limit of the standard set by Decree 41/2012, which sets the limit at 20 %. It was, therefore, across all groups data with a small dispersion and, therefore, the data obtained from the following samplings could be statistically evaluated directly.

In the case of the free acidity, at the beginning of the experiment, the groups F, O and C showed data with a small dispersion. Their average values according to groups were: F – 18.17 ± 0.29 mEq/kg, O – 18.33 ± 3.79 mEq/kg and C – 19.67 ± 4.25 mEq/kg.

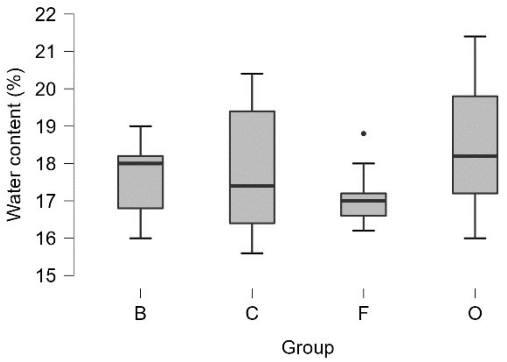

Figure 5. Boxplot graph of water content in control and experimental groups

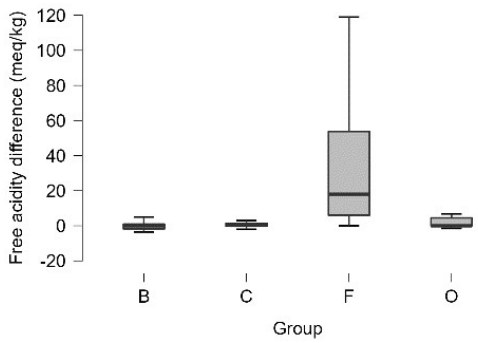

Figure 6. Boxplot graph of free acidity difference in control and experimental groups

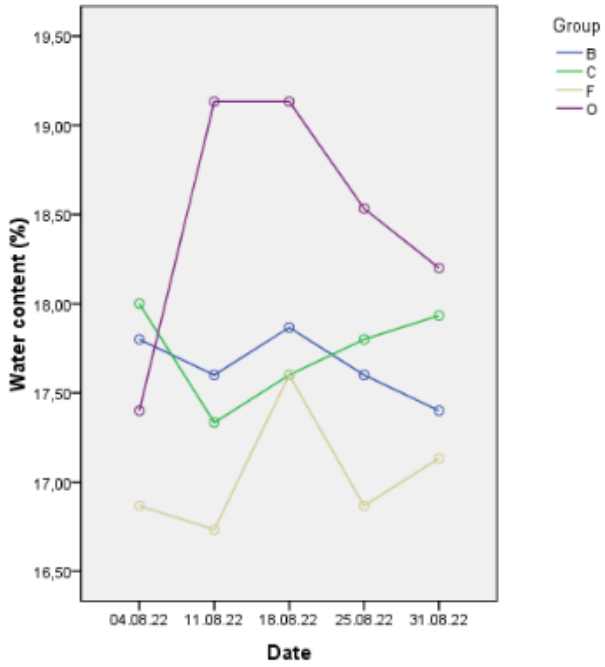

Figure 7. Dynamics of water content values during the experiment

The average value for group B (16.33 ± 0.58 mEq/kg) was further away from the other groups. In addition, it maintained a low value during the entire course of the experiment. Therefore, the difference between the measured values after the application of the treatment in the individual sampling dates, and the original value, with which the bee colony entered the experiment even before the administration of the drug, were calculated to adjust the data for the objectivity of comparing the increase in the free acidity. The values of the differences in the free acidity were statistically processed (Figures 8 – 13).

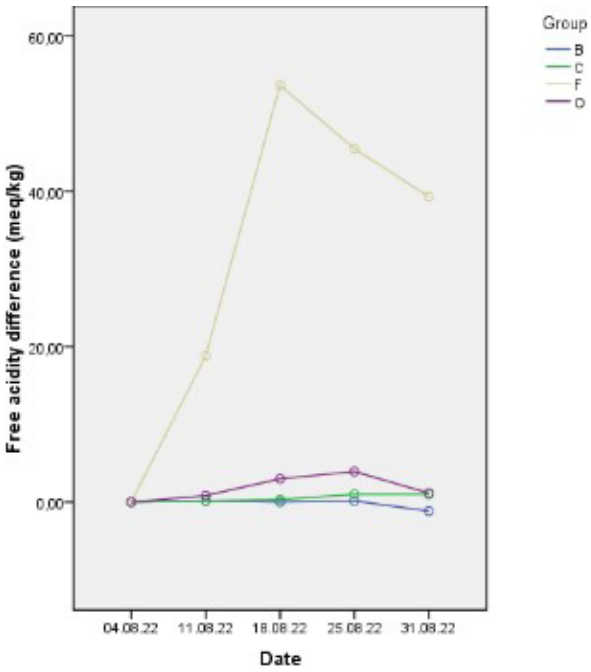

Figure 8. Dynamics of free acidity difference during the experiment

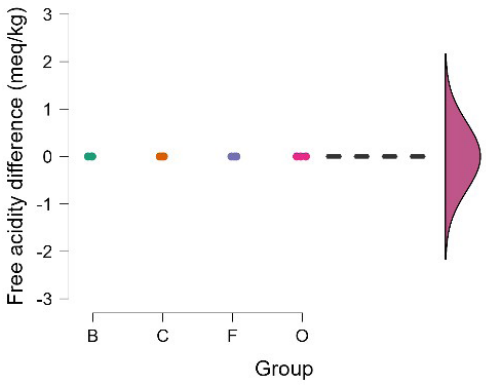

Figure 9. Free acidity difference at the beginning of the experiment (1st collection)

The results pointed to the fact that although the acidity of honey in the B group did not show a significant difference during the experiment, compared to the control group ($p = 0.769$) and in the O group it showed only a slight increase ($p = 0.416$) compared

to the control, the F group showed a significant difference in the increase of values during the experiment compared to the control group (p = 0.015). One week after the introduction of vaporizers, FA caused an increase in the free acidity value of honey by 18.83 ± 17.82 mEq/kg. In the second week after inserting and refilling the vaporizer, the increase in free acidity reached 53.67 ± 56.66 mEq/kg compared to the original level at the beginning of the experiment. Subsequently, the vaporizer was removed from the hive and the difference began to slowly decrease. At the end of the experiment, two weeks after removing the vaporizer, mean value of free acidity was 57.50 ± 25.19 mEq/kg. During the application of towels with OA glycerine solution there was only slight increase in the second and third weeks during the application. These differences in values compared to the original value were 3.00 ± 3.61 and 3.93 ± 3.52 mEq/kg, respectively. At the end of the experiment, the difference was only 1.17 ± 3.79 mEq/kg.

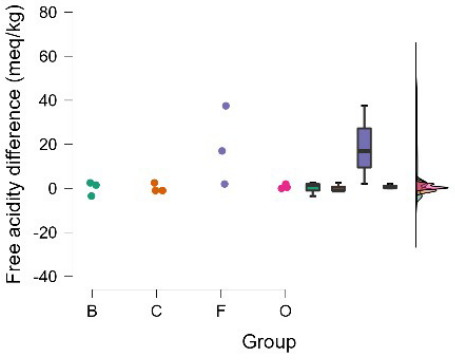

Figure 10. Free acidity difference during the 2nd collection

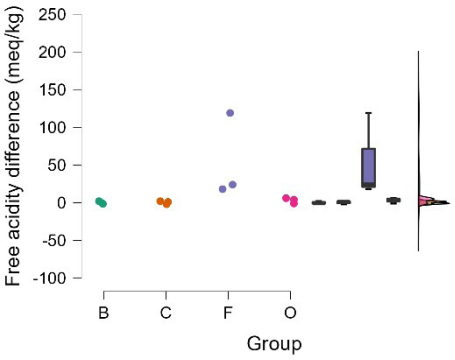

Figure 11. Free acidity difference during the 3rd collection

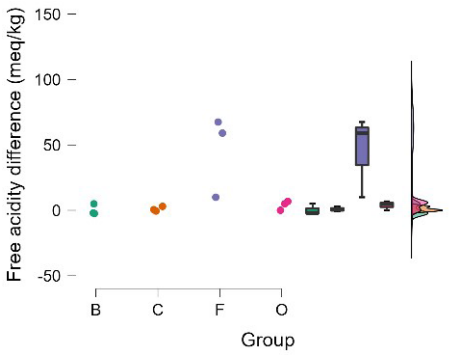

Figure 12. Free acidity difference during the 4th collection

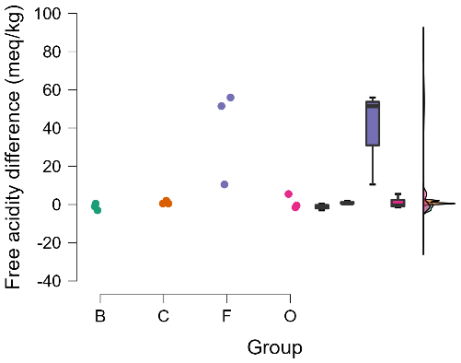

Figure 13. Free acidity difference during the 5th collection

To determine the dependence of the free acidity on the content of water in honey, the data were subjected to a correlation analysis. Its results showed a strong positive correlation between water content and free acidity values for the control group ($r = 0.912$, $p < 0.001$), a moderately strong correlation for the F group ($r = 0.788$, $p < 0.001$) and the O group ($r = 0.789$, $p < 0.001$). In the B group, only a weak positive correlation ($r = 0.397$, $p = 0.143$) was observed.

DISCUSSION

Summer treatment of bee colonies against varroosis represents an increasingly topical problem. The probability of an increase in residues of synthetic acaricides in bee products and the possibility of resistance leads many beekeepers to the idea of using an alternative, either in the form of FA, OA, or

essential oils. Effectiveness of the treatments vary and is influenced by numerous factors. Sabahi *et al.* (2020) compared the application of thymol in glycerine and OA in glycerine against varroosis and found the effectiveness of 92.4 % and 79.0 %, respectively. In term of the treatment by vaporizer Nassenheider, long-term observations (from 2011 to 2019) indicated the 60 %-FA effectiveness (51.9 ± 27.8 %) against *Varroa destructor* and higher FA efficiency in single brood chamber hive compared to hive with two brood chambers (Steube *et al.*, 2021).

No direct maximum residue limit (MRL) values have been established for FA. Therefore, when evaluating the quality of honey after treatment, the free acidity is considered. This varies depending on the evaporation conditions, concentration and dose of FA, as well as the time that elapses from the end of the treatment to the honey collection. A study dealing with the effect of FA on the acidity of honey in Switzerland reported that the autumn application of 130 ml of 70 % FA over 7 days does not cause a significant increase in acidity, provided that the honey is collected the following season (Bogdanov *et al.*, 2001).

Spring treatment significantly reduces the population of the mite during the summer and in the fall (Přidal and Svoboda, 2012a). Experimental model in this research was based on the conditions, when the beekeeper applies FA in the summer to protect the winter generation of bees from mites and, at the same time, counts on the last honey collection after the application of the acid. At the same time, higher, maximum technically possible but practically realistic, doses of 60 % FA were used. The evaporation achieved 21 – 26 g FA per day compared to 13 g in the Swiss study. The ideal recommended dose for our conditions is 7 g/day (Krämer, 1982). The dose was chosen due to the possibility that the beekeeper can rely on the autoregulation of the Nassenheider Professional® vaporizer and may refill the storage container. This is, therefore, the worst possible treatment scenario. The results showed that even in such a case, after two weeks there was not a sufficient decrease in the free acidity values. Two weeks after application, the honey still reached the mean value of free acidity (57.5 ± 25.19 mEq/kg), which is over the legislative limit. In the case, when FA was administered as an emergency spring treatment and honey was collected in the summer, there was a significant increase in the free acidity, which was evident (although not statistically significant) compared to the autumn treatment (Bogdanov *et al.*, 2001). It can, therefore, be assumed that summer treatment will be more problematic in relation to the acidity of honey. The other two studies were devoted to a model, where FA was administered in the fall and its content was monitored in the stock until the spring of the following year. In both cases, a large increase in its content was observed immediately after application and a gradual decrease until spring, when its content reached values like those originally recorded before application (Stoya *et al.*, 1986; Capolongo *et al.*, 1996). Large fluctuations in the free acidity values in honey, as well as the different effectiveness of FA related to the intensity of evaporation (Přidal and Svoboda, 2012b), however, lead beekeeping practice to the increasingly frequent use of OA.

In our experiment, OA was applied by contact using a towel soaked in a glycerine solution of OA. Several authors have addressed the OA residues in honey or in carbohydrate stores after the application of this active substance (Mutinelli *et al.*, 1997; Del Nozal *et al.*, 2000; Bernardinie and Gardi, 2001, Bogdanov *et al.*, 2001). They failed to record a significant increase in OA content. Likewise, in this experiment, the free acidity values were not significantly changed during the entire application period. At the end of the experiment, with the constant presence of the carrier, even free acidity dropped to the original value.

Knowing the effect of OA and FA on the quality of honey is also important because some authors of studies consider using a combination of these two substances, because using together they show higher efficiency. At the same time, however, they also report a significant decrease in adult bee counts after such treatment (Pietropaoli and Formato, 2022).

In our experiment, the essential oils in the Bisanar® preparation did not have a significant effect on the free acidity in honey. Studies dealing with the determination of residues of these substances focus more on their influence on sensory properties (Tüshaus, 1993; Bogdanov *et al.*, 1999). In Switzerland, due to the significant influence on taste, they proceeded to introduce the MRL of thymol in honey at 0.8 $mg.kg^{-1}$. According to the FAO, this substance is considered safe from the point of view of consumer health up to the concentration of 50 $mg.kg^{-1}$. At the same time, a person can detect a change in taste from a concentration of 1.1 – 1.3 $mg.kg^{-1}$ (Bogdanov *et al.*,

1999). Even in the case of essential oils, it is necessary to know their effect on the acidity of honey, as scientists are also testing the combination with OA for these active substances (Toomemaa, 2019). Thymol has the strongest effect on honey taste (Bogdanov *et al.*, 1999). Change of the natural honey aroma by essential oil is not beneficial. The test persons marked the astringent and "medicinal" taste of honey with certain concentration of thymol, camphor, or menthol (Bogdanov *et al.*, 1999).

CONCLUSIONS

Summer treatment with FA causes a significant increase in the free acidity value in honey. It is necessary to focus on the development of methods of treatment product application with FA, which would minimize the risks to the bee colony and the quality of honey during summer application. According to the results, it is recommended to harvest honey not sooner than 3 weeks after removing the vaporizer from the hive.

Summer treatment with a glycerine solution of OA does not significantly affect the acidity of honey. From this aspect, it can therefore be considered as a suitable active substance for summer treatment against varroosis. The summer treatment of bee colonies with Bisanar® did not cause an increase of free acidity in honey. In the last both cases (i.e., the treatment by OA or essential oils against varroosis) the high efficiencies against *Varroa destructor* were found.

Moreover, a positive correlation between the increase in the free acidity in honey and its water content was found. Correct collection of matured, capped honey can increase the quality of the honey put on the market.

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AUTHOR CONTRIBUTIONS

Conceptualization: Staroň, M., Kňazovická, V.
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Investigation: Staroň, M., Kňazovická, V., Gasper, J.
Data curation: Staroň, M., Kňazovická, V.
Writing-original draft preparation: Staroň, M.
Writing-review and editing: Kňazovická, V.
Project administration: Staroň, M.

All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Bernardini, M. & Gardi, T. (2001). Influence of acaricide treatments for varroa control on the quality of honey and beeswax. *Apitalia*, 28(7–8), 21–24.

Bogdanov, S., Charrière, J. D., Imdorf, A., Kilchenmann, V. & Fluri, P. (2002). Determination of residues in honey after treatments with formic and oxalic acid under field conditions. *Apidologie*, 33(4), 399–409. https://doi.org/10.1051/apido:2002029

Bogdanov, S., Kilchenmann, V., Fluri, P., Bühler, U. & Lavanchy, P. (1999). Influence of organic acids and components of essential oils on honey taste. *American Bee Journal*, 139(1), 61–63.

Capolongo, F., Baggio, A., Piro, R., Schivo, A., Mutinelli, F., Sabatini, A. G., Colombo, R., Marcazzan, G. L., Massi, S. & Nanetti, A. (1996). Trattamento della varroasi con acido formico: accumulo nel miele e influenza sulle sue caratteristiche. *L'Ape Nostra Amica*, 18, 4–11.

Del Nozal, M. J., Bernal, J. L., Diego, J. C., Gómez, L. A., Ruiz, J. M. & Higes, M. (2000). Determination of oxalate, sulfate and nitrate in honey and honeydew by ion-chromatography. *Journal of Chromatography A*, 881 (1–2), 629–638. https://doi.org/10.1016/S0021-9673(00)00271-5

IHC. (2009). *Harmonised Methods of the International Honey Commission*. B. m.: B. v. Retrieved from: www.ihc-platform.net

Kary I. (1987). *Untersuchungen zur Rückstandsproblematik in Bienenhonig im Rahmen der Varroatosebekämpfung*. Dissertation, Justus-Liebig-Universität Giessen, Giessen, Deutschland.

Krämer, K. (1982). Ameisensäure als Bekämpfungsmittel der Varroa-Milbe im Bienenvolk II. *Die Biene*, 118(2), 55–58.

Mutinelli, F., Baggio, A., Capolongo, F., Piro, R., Prandin, L. & Biasion, L. (1997). A scientific note on oxalic acid by topical application for the control of varroosis. *Apidologie*, 28(6), 461–462.

Pietropaoli, M. & Formato, G. (2022). Formic acid combined with oxalic acid to boost the acaricide efficacy against *Varroa destructor* in *Apis mellifera*. *Journal of Apicultural Research*, 61(3), 320–328. https://doi.org/10.1080/00218839.2021.1972634

Přidal, A. & Svoboda, J. (2012a). *Spring treatment of honey bee colony with formic acid limits intensity of varroosis in the autumn. I.* Seminár včelárskej praxe (s. 15–20). Liptovský Hrádok: Ústav včelárstva Liptovský Hrádok. ISBN 978-80-89418-16-9.

Přidal, A. & Svoboda, J. (2012b). *The critical limit of air temperature within in the evaporation of formic acid. I.* Seminár včelárskej praxe (s. 4–14). Liptovský Hrádok: Ústav včelárstva Liptovský Hrádok. ISBN 978-80-89418-16-9.

Sabahi, Q., Morfin, N., Emsen, B., Gashout, H. A., Kelly, P. G., Otto, S., Merrill, A. R. & Guzman-Novoa, E. (2020). Evaluation of dry and wet formulations of oxalic acid, thymol, and oregano oil for *Varroa* mite (*Acari: Varroidae*) control in honey bee (*Hymenoptera: Apidae*) colonies. *Journal of Economic Entomology*, 113(6), 2588–2594. https://doi.org/10.1093/jee/toaa218

Steube, X., Beinert, P. & Kirchner, W. H. (2021). Efficacy and temperature dependence of 60 % and 85 % formic acid treatment against *Varroa destructor*. *Apidologie*, 52(3), 720–729. https://doi.org/10.1007/s13592-021-00859-5

Stoya, W., Wachendörfer, G., Kary, I., Siebentritt, P. & Kaiser, E. (1986). Ameisensäure als Therapeutikum gegen Varroatose und ihre Auswirkungen auf den Honig. *Deutsche Lebensmittel-Rundschau*, 82(7), 217–221.

Toomemaa, K. (2019). The synergistic effect of weak oxalic acid and thymol aqueous solutions on *Varroa* mites and honey bees. *Journal of Apicultural Research*, 58(1), 37–52. https://doi.org/10.1080/00218839.2018.1486695

Tüshaus, M. (1993). *Gaschromatographischer und sensorischer Thymolnachweis in Bienenhonig zur Beurteilung der Rückstandsproblematik bei der Varroabekämpfung mit ätherischen Ölen*. Dissertation, Universität Hohenheim.

Vyhláška č. 106/2012 Ministerstva pôdohospodárstva a rozvoja vidieka Slovenskej republiky z 9. marca 2012, ktorou sa mení vyhláška MPRV SR č. 41/2012 Z. z. o mede [Decree No. 106/2012 of the Ministry of Agriculture and Rural Development of the Slovak Republic of 9 March 2012 amending the Decree of the Ministry of Agriculture and Rural Development of the Slovak Republic No. 41/2012 Coll. on Honey].

Vyhláška č. 41/2012 Ministerstva pôdohospodárstva a rozvoja vidieka Slovenskej republiky z 26. januára 2012 o mede [Decree No. 41/2012 of the Ministry of Agriculture and Rural Development of the Slovak Republic of 26 January 2012 on honey].