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IMPROVING THE FORMIC ACID-BASED FORMULAS USED IN VARROOSIS CONTROL BY BROOD BRUSHING PROCEDURE

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Abstract

The paper aims to present some preliminary results regarding the effectiveness of different lower concentration of formic acid formulas, used by brushing procedure, in killing the varroa mites (Varroa destructor) which are found in the reproductive phase in the capped brood. To perform the experiments, honeybee capped brood combs from untreated colonies were collected and treated in two experimental groups with different dilutions (20%, 30%, 40%, 50%) of concentrated formic acid (85%) used in water-based and alcohol-based dilutions. The measurements were focused on the evaluation the varroa mite mortality, as response variable, at 72 hours from the treatment application. Out of the obtained results one could remark that the mortality of mites increased as the concentration of formic acid increased in different formulas. Highly significant differences were established between the two experimental groups as well as between the mortality of different categories of varroa. The results clearly show that the use of formic acid is very effective in varroosis control also when used in lower concentrations (30-40%) by brushing procedure. The results also show that new formulas could be further optimised by setting up a standard protocol to evaluate the critical stages of mites inside brood and their vitality, which are affected following the application of treatments.

***Key words**: brushing procedure, capped brood, formic acid, honeybee, varroa, varroosis.*

INTRODUCTION

The recent efforts to control varroosis (*Varroa destructor*) are more and more focused on different alternative approaches (Van der Steen & Vejsnæs, 2021; Vilarem et al., 2021; Roth et al., 2020), taking into account the need to practice a sustainable beekeeping which protect honeybees as well as their environment and products. In the frame of these approaches, the biotechnological methods combined with the application of treatments based on soft, organic acaricides, have a special place (Büchler et al., 2020; Lodesani et al., 2019; Siceanu et al., 2019; Gregorc et al., 2017; VanEngelsdorp et al. 2008; Amrine & Noel, 2007; Fries, 1991). Generally, all these treatments aim to lower the infestation level before critical moments in the active season in parallel with taking the measures to avoid the hive contamination and the resistance phenomenon in the mite population (Büchler et al., 2019; Lodesani et al., 2019; Sara Hernández-Rodríguez, et al. 2021). In the last years, it was developed an alternative strategy to control varroa mite infestation by using a biotechnical procedure of brushing the capped brood with concentrated organic volatile acids (Siceanu et al., 2021). This procedure proved to be very effective in killing the mites found in the reproductive phase. In a pilot study (Căuia & Căuia, 2022), using the formic acid of 65% concentration by brood brushing in spring applications, the infestation level in late summer was drastically reduced. This is the period of the year in temperate climate when, naturally, the varroa mite population increases and poses serious risks for honeybee colony health for the next beekeeping season. Taking into account some practical observations on the immediate effectiveness of these targeted treatments on varroa mite mortality an important question arose on the effectiveness of organic volatile acids when used in lower concentration. The use of lower concentrations of formic acid could also offer a better protection and manipulation of the brood combs, but the whole colony too, allowing the reintroduction of the treated combs in the colony just after the treatment application. No less important is the fact that a softer formula could offer a better protection for beekeepers, diminishing thus the potential hazardous risks of treatment product manipulation. Thus, the aim of this research work was to preliminary test the effectiveness of different lower concentration of formic acid formulas, used by brushing procedure, in killing the varroa mites which are found in the reproductive phase in the capped brood

MATERIALS AND METHODS

Experiment design.

1. ***Biologic material.*** To perform the experiments, there were used honeybee capped brood combs collected in the active seasons of the 2020-2021 years. The combs originated from untreated colonies (*Apis mellifera carpatica*), belonging to an experimental apiary of the Honeybee Genetics and Breeding laboratory in the frame of the Institute for Beekeeping Research and Development located in Bucharest. The honey bee colonies that provided varroa infested brood were managed in Dadant hives on 10 frames. These colonies were not treated one season before (in the end of summer and in the autumn of the previous year) in order to have a higher level of infestation with varroa mite in the experimental units, so to increase the probability to easily find infested cells to facilitate the measurements.

2. ***Experimental groups.*** For the purpose of this study there were established two experimental groups (EG1 and EG2) and different dilutions (20%, 30%, 40%, 50%) of the concentrated formic acid (85%). The two experimental groups were set up to test two types of dilutions, based on distilled water (EG1), respectively on concentrated alcohol (ethanol) of 97% (EG2). The main information regarding the experimental design is presented in the table 1. A total number of 25 capped brood combs were treated and evaluated, with 2-4 combs on each experimental variant. This biologic material was collected out of 10 colonies, at different time intervals in July-August period when level of infestation naturally increases. The combs were selected to have brood in the pupae stage so that to identify the state of varroa mites (live or dead) in different developmental stages in the treated brood. The individuals were identified based on their morphologic characteristics (Mondet et al., 2020; Rosenkranz et. al 2010).

Table 1. The experimental design to test the impact of different concentrations of the formic acid formulations on varroa mite in the capped brood, following the treatments by brushing procedure application.

Experimental groups	Active substance	Solvent	Concentration of formic acid in the treatment formulas (%)	No of treated combs by brushing procedure
Variants of EG1				
1.1	Formic acid 85%	Distilled water	50	2
1.2	Formic acid 85%	Distilled water	40	4
1.3	Formic acid 85%	Distilled water	30	4
1.4	Formic acid 85%	Distilled water	20	4
Variants of EG2				
2.1	Formic acid 85%	Ethanol 97%	50	2
2.2	Formic acid 85%	Ethanol 97%	40	2
2.3	Formic acid 85%	Ethanol 97%	30	3
2.4	Formic acid 85%	Ethanol 97%	20	4

3. ***Treatments.*** The treatments were applied on capped brood combs, using the brushing procedure as explained in the literature (Siceanu et al., 2021).

Immediately after treatments application, the treated combs were returned into the origin colonies for 72 hours, in order to better highlight the effect of treatments on the varroa mite mortality. This time interval was chosen to better evaluate the varroa mortality, as by previous observations (Siceanu et al., 2021) the level of mortality can be influenced by time, concentration in volatile substances or the degree of exoskeleton sclerotization of the mite, as for example, the adult mites are the most resistant to this type of treatment. The chosen time interval could offer also supplementary information about the status of each individual, their vitality and other information regarding the reproduction, e.g., the presence of eggs or protonymphs which can reflect an interruption of reproductive process.

4. ***Measurements***. After 72 h since the treatment application, the combs were collected again and examined under stereomicroscope (Olympus SZ61), at 6.7X-45X magnifications, in order to easily identify the infested cells and different categories of varroa mites.
The capped cells were opened with tweezers, cell by cell, in more rows, to increase the probability to identify the infested cells. Generally, at least 200 cells were checked out on each treated comb (Dietemann et al., 2013).
The measurements were focused on the evaluation the mite mortality as response variable, by counting the total number of found mites which was split into live individuals (including those with a low vitality) and dead individuals for every developmental stage. The statistical analysis was performed using the NCSS 2021 v21.0.2 software, following the literature recommendations (Sandu, 1995).

RESULTS AND DISCUSSIONS

By previous researches (Siceanu et al., 2021) it was shown that the capped brood treatment with formic acid (65%, respectively 85%) by using the brushing procedure has a high effectiveness in killing both, the adult and juvenile stages of varroa mite (Ave.= 90.48%, respectively 92.64%) found inside cells, at 24 hours evaluations after treatment application. By lowering the concentration of formic acid in different formulation using various dilutions and solvents there were obtained different results in terms of the effectiveness on varroa mite mortality evaluated at 72 hours after treatments application (Table 2).

Table 2. The results regarding the effectiveness of different concentrations of formic acid formulations on the mortality of varroa in capped brood using the brushing procedure

Experimental groups	Total mites (no.)	The number of dead mites/The number of total mites (% of mortality)					Mortality of mites (%)
		Foundress female	*Adult male*	*Protonymph*	*Deutonymph*	*Adult daughter*	
EG 1. Variants of water-based formulas							
1.1. **50% FA**	210	71/86 (82.5)	6/7 (85.7)	83/85 (97.6)	26/31(83.9)	0/1 (0)	88.6
1.2. **40% FA**	172	65/80 (81.3)	3/ 4 (75)	42/44 (95.5)	26/28 (92.9)	7/16 (43.8)	83.1
1.3. **30% FA**	214	27/85 (31.8)	1/12 (8.3)	46/48 (95.8)	32/46 (69.5)	8/23 (34.8)	53.3
1.4. **20% FA**	207	23/113 (20.4)	0/9 (0)	37/50 (74)	15/26 (57.7)	1/9 (11.1)	36.5
Average	**200.1**	**186/364 (51.1)**	**10/32 (31.2)**	**208/227 (91.6)**	**99/131 (75.5)**	**16/49(32.6)**	**64.6**
EG2. Variants of ethanol-based formulas							
2.1. 50% FA	121	66/71 (93)	1/1 (100)	40/40 (100)	5/5 (100)	4/4 (100)	95.9
2.2. 40% FA	56	35/38 (92.1)	1/1 (100)	10/10 (100)	7/7 (100)	0/0 (-)	94.6
2.3. 30% FA	198	75/85 (88.2)	5/9 (55.6)	54/56 (96.4)	30/30 (100)	13/18 (72.2)	89.4
2.4. 20% FA	201	62/104 (59.6)	12/18 (66.7)	35/38 (92.1)	19/21 (90.5)	10/20 (50)	68.7
Average	**144**	**238/298 (79.8)**	**19/29 (65.5)**	**139/144 (96.5)**	**61/63 (96.8)**	**27/42 (64.3)**	**84.0**

Out of these results one can notice that the mortality of mites increases as the concentration of formic acid increases in different formulas (Figure 1). Additionally, in the alcohol-based dilution formulas a higher mortality of varroa mite was registered as compared with water-based dilution ones, in all experimental variants. One explanation could be the fact that alcohol amplify the vaporisation process of the formula and/or together with formic acid could have cumulative effects on varroa mite mortality.
Applying the MANOVA statistical analysis highly significant differences were established between the two experimental groups (F ratio=20.9, P= 0.010, α=0.05), illustrated in the figure 2. Highly significant differences were also established between the mortality of different categories of mites (F ratio=12.74, P= 0.015, α=0.05) as a result of different treatments, the differences being well illustrated in the Figures 2 and 3.

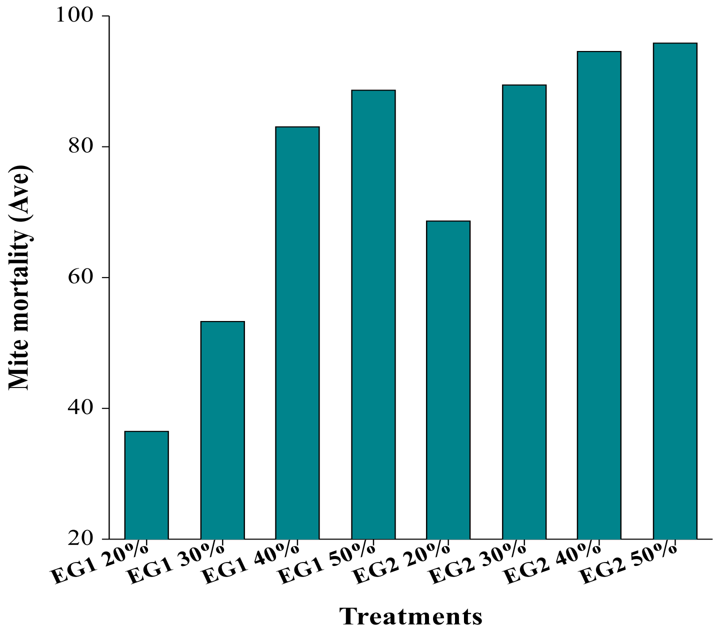

Figure 1. The effect of different treatments in the experimental variants on total varroa mite mortality.

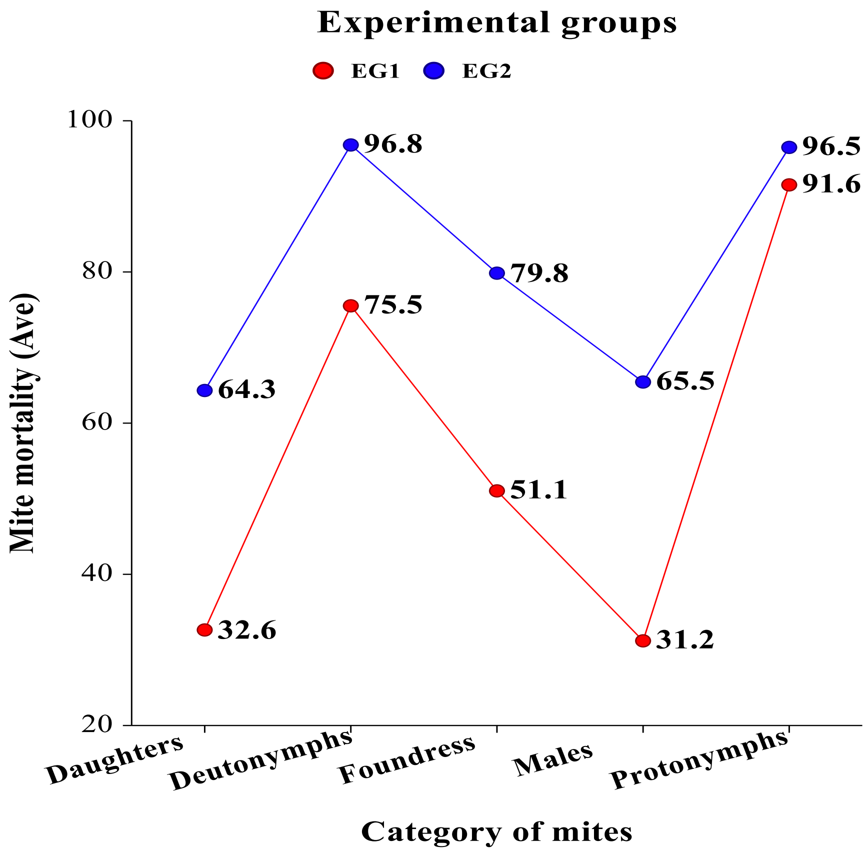

Figure 2. The effect of the treatments in the experimental groups on different categories of varroa mite.

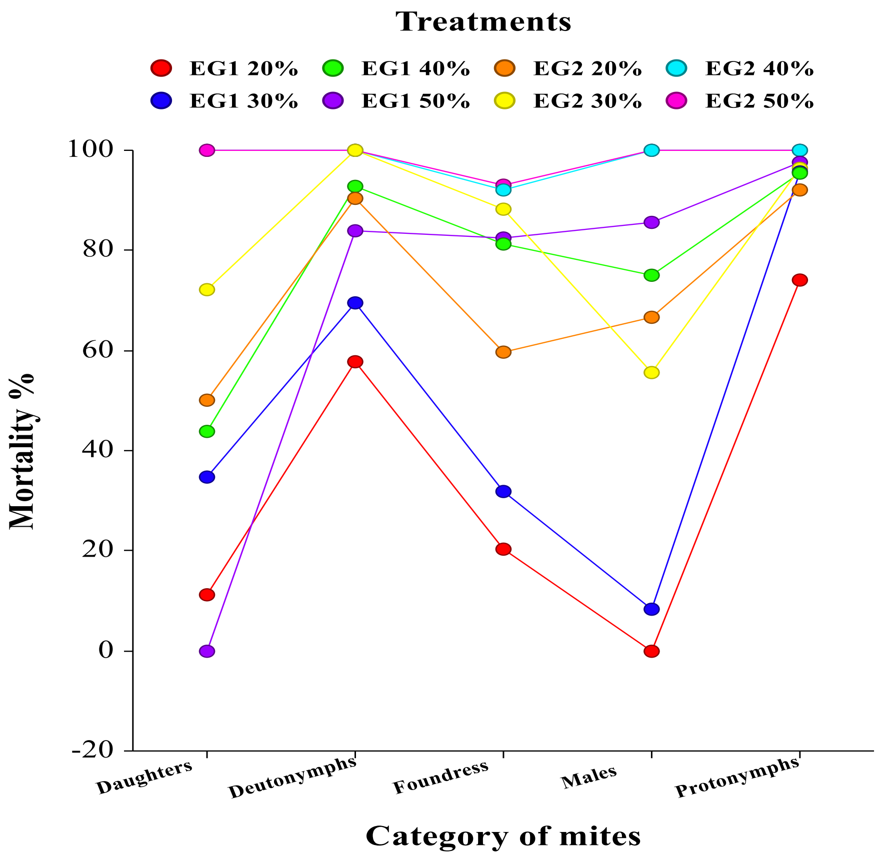

Figure 3. The effect of different treatments on each category of varroa mite found in brood.

The highest varroa mite mortality was registered in the treatments based on formic acid of 50% concentration (Ave.=88.6% in water-based formula; Ave.=95.9% in alcohol-based formula), decreasing slowly in the case of formic acid of 40% concentration (Ave.=83.1% in water-based formulas; Ave.=94.6% in alcohol-based formulas), and registering the lowest values in the treatments with formic acid of 20% concentration (Ave.=36.5% in water-based formula; Ave.=68.7% in alcohol-based formula). It is possible that the alcohol-based formulation may lead to the obtaining of ethyl formate which is an ester formed when ethanol is combined with formic acid, being an organic substance that occurs in nature. It is not known if this substance was produced in the different formulations at the treatment moment, which is the amount produced or if it would have effects on varroa mite. This topic would require special investigations.

Regarding the effect of treatments on different categories of varroa mite, as can be seen out of figures 2 and 3, the most vulnerable stages are the protonymphs and deutonymphs. This can be explained by the lack of exoskeleton sclerotization, but also by affecting their feeding which is dependent by the presence of foundress females with normal vitality. Out of the observations done on live varroa mite, including the foundress females, the most parts of these individuals were found in a low vitality state especially in the 30-50% dilutions and in many cases, the most fragile forms (males, protonymphs) were not anymore identified in the cells according with the reproductive pattern (Mondet et al, 2020) or they were found in an advanced degradation status. The low vitality means individuals with a visible lower mobility or being in a morbid status. To simplify the testing of the treatment's effectiveness performed on brood, the presence of eggs and viable protonymphs at 72 hours evaluation moment could be a better indicator of reproducing mites, which to be used in further evaluations. Taking into account the high mortality of protonymphs even in lower concentrations one can suggest that the obtained data do not reflect totally the minimal limit of optimal concentration of formic acid-based formulations, hence the preliminary nature of these researches. Out of the obtained results only a limited number of adult males were identified the most of them being included in protonymphs category, where the mortality was very high both in water-based and alcohol-based formulas. As the males' presence and vitality is very important for mating, their mortality is another important indicator for the effectiveness of treatments evaluation. In these conditions, a protocol for evaluation the vitality of varroa mite in the capped brood is very important to be established, to correctly estimate the impact of different treatments performed on brood. By brood treatment with low concentrations of formic acid one can improve the use of brushing procedure in organic and conventional beekeeping, taking into account that in the treatments applied on the whole colony the lowest concentration of formic acid used is 50% (Amrine et al. 2007).

CONCLUSIONS

The results of this study offer useful data on the effectiveness of formic acid in lower concentrations on varroa mite mortality, when applied by brushing procedure. Being about a targeted procedure, the concentration decreasing of formic acid used is an important goal for the optimisation of new formulas and their application on capped brood whenever is necessary in the management of the apiaries in active season as part of the sustainable control of varroosis, both in organic and conventional beekeeping.

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