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Preliminary Researches Regarding the Effectiveness of the Formic Acid Treatment on Varroa (*Varroa destructor*) Found in the Artificially Decapped Bee Brood

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Abstract: The objective of the study was to establish the effect of formic acid on varroa (*Varroa destructor*), inside the capped brood cells, artificially decapped. The experiments were carried out in 2017-2018 on honeybee colonies infested with varroa (*V. destructor*), in a research apiary belonging to the Institute for Beekeeping Research and Development in Bucharest. The decapping method in the present researches used the decapping fork to scrape the capped comb, without affecting the brood, in order to open it for an effective treatment. The combined treatment method was applied on honeybee colonies as a whole, as well as on brood combs, without bees, put in a special treatment box. The researches were focused on establishing the mortality level of various stages of varroa in artificially decapped brood, in normal colony and separately, as well as to make observations on the effect of formic acid on viability of capped bee brood, artificially decapped. The results show a high mortality of varroa, especially the protonymphs and deutonymphs stages (over 80%). The main conclusion is that the brood decapping method combined with formic acid treatment could be a useful technique to control varroa infestation, both in brood and honeybees, shortening strongly the treatment duration as compared to the usual treatments, increasing the efficacy of treatment by cutting the life cycle of varroa in brood.

Key words: Varroa (*Varroa destructor*), honeybee (*Apis mellifera*), brood, artificial decapping, formic acid.

1. Introduction

Varroa destructor Anderson and Trueman 2000 (Acari: Varroidae) is the ectoparasitic mite of the honey bee, *Apis mellifera* L. (Hymenoptera: Apidae) which causes important damages in beekeeping. Since its introduction into Europe and Americas, numerous researches have been carried out in order to understand its biology and to find better solutions to control it [1].

Its reproduction characteristics connected to capped brood lead to a general poor efficacy of actual treatments which are limited to kill mites on adult bees.

The need to cut the life cycle is ever increasing, also taking into account the new findings which emphasize the destructive capacity of varroa that feeds primarily on fat body [2], which affects the health status of honeybees in all developing stages. The specific treatments are very important in varroosis as the non-treated colonies register gradual decreasing of colony strength, being followed by its death in 2-3 years from initial infestation [1, 3].

Breeding for resistance to *V. destructor* is regarded as a viable long-term solution [4, 5], but this involves very complex selection activities, on a large scale, and death risk is high if the colonies are not regularly checked against the infestation threshold and treated. Drone removal combined with drone trapping followed sometimes by one acaricide treatment was found as the solution to efficiently control varroa infestation in the integrated pest management [6].

The importance of the treatment's quality is also crucial with respect to bee products contamination and increasing varroa resistance to the synthetic

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acaricides.

The actual treatments with organic substances are often complex and differ from one region to another [1, 7-9], depending on how long the brood rearing period is or other key moments as beginning of winter bee rearing period.

Organic substances can be very efficient in killing the organisms, but usually their dose is an important issue as it can be easily harmful for host and parasite at once.

As it is already very well known, formic acid (FA) is an organic substance, found in nature as a defending molecule, in different organisms (e.g., ants, bees, jellyfish, scorpions, beetles, plants), as well as in atmosphere by natural biodegradation processes, occurring also naturally as traces in some bee products (honey). FA can be produced artificially by chemical synthesis, being used as preservative and antimicrobial agent for livestock feed, in industry (textile, leather, rubber, production of cleaning products) and in chemistry laboratories. In beekeeping it is a very effective substance for mite control treatment, even if the action (suffocation or respiratory inhibition) is less understood [1]. Another very important advantage of this substance is that it does not contaminate the combs or other bee products. Some disadvantages are connected with its corrosive effects on hive ferrous elements and the irritation action for the applicator, its handling being a very important issue.

Being a highly volatile substance, FA is very efficient for controlling varroa [10-12], filling rapidly any space in the beehive. Even if the caped brood cells are not air-tight and the FA can enter through the caps, killing off also the mites inside [1, 13], the caps are an important barrier in the diffusing process.

Because FA is a volatile substance whose diffusion is temperature and humidity dependent, the level of vapors is hard to be kept constant in time, which is why it is used when the temperature outside the hive is in the range of 12-25 °C. Following this specificity, the under-dosing and overdosing can be problematic, especially because the usual period of one treatment is relatively long. The usual treatments are recommended in brood right time, especially in July-October, but some are recommended also in spring.

Actually, there are different methods used, for the varroa treatment with FA [14-20] with various degrees of effectiveness, most of them being completed by classical treatments for a better control. The concentration (60%-85%), quantity (50-120 mL) and application methods (pads impregnation, gel formulations, liquid dispensers) as well as their place in the colony are quite diverse. As some researches show [21], the more irregular the evaporation rates of FA are, the higher is the toxicity of the treatment, causing the mortality of developing eggs and brood. In higher concentration it is toxic also for adult bees and queens [22, 23].

The treatment duration, which is relatively long, has a lot of drawbacks as queen mortality, depletion of colony, swarming stimulation, reduction of productivity [24]. Thus, in the nowadays practice, one treatment, that is recommended to last 7-10 d, can have a very negative impact on the bee colony activity and development.

A short term (17 h) fumigation with FA (50%) was also investigated [25] on honeybee colonies, the results showing that there is a good effectiveness in killing varroa inside the capped brood in the same time with those found on adult bees (> 60%).

The new idea was explored in some practices, by separating the brood frames, using a special box—Formico box [26] and treating them with FA 70-100 mL for 2 h in order to evaporate 25-30 g of FA as a minimum quantity for a good effectiveness of substance on mites inside the cell. In this practice, the prepared treatment boxes with brood combs are isolated with plastic bags and let in the sun for a better evaporation. The method seems to be effective, but a total loss of the uncapped brood was mentioned as well as a partial loss of the hatching bees which are the most vulnerable.

Developed in 1996, the artificially brood decapping method (ABDM) is a special method created to understand better the biology of brood and varroa, as well as to be combined with treatments for varroa controlling in brood [27].

By the recent researches [28], the method was improved in order to increase the rapidity of the decapping action, to be easily used by beekeepers, in order to control varroa in combination with FA, in one treatment, to cut rapidly the life cycle of varroa before reaching the damaging threshold.

Moreover, the method can be used by itself to stimulate the honeybees to remove the mites from infested brood in the so called Varroa Sensitive Hygiene/Suppressed Mite Reproduction (VSH/SMR) behavior and to decrease the reproduction success related to the escaping behavior of mite from the artificially decapped cells [29].

Having in view the state of the art of the varroa control by FA, the aim of the present study was to improve significantly the varroa treatment by combining two actions: the application of the ABDM followed by a soft and very short-term treatment based on FA in order to reduce significantly the number of varroa found in brood.

By the application of the ABDM, all stages of varroa in brood will be very well exposed to active substance, increasing its efficacy and reducing the time of the treatment as well as the number of treatments per year. In the light of these findings, this type of treatment could be applied both in honeybee colonies as well as in special boxes designed for brood comb separation.

Taking into account the proposed new combined method and treatment to control varroa (ABDM + FA treatment), one of the goals of the experiments was to establish its efficacy in terms of dead varroa in different development stages, in the colony and separately on brood frames in special boxes, in a short-term application, using different concentrations.

The new combined treatment method can be successfully used, both in organic and conventional beekeeping as FA treatment is a residue free one. Some important observations and conclusions, raised from the practical use of this combined method, were finally drawn.

2. Materials and Methods

The experiments were performed in a research station apiary, belonging to the Institute for Beekeeping Research and Development in Bucharest, in two periods: September-October 2017 and July-September 2018. To perform the experiments, untreated honeybee colonies, naturally infested, were used in order to increase the varroa infestation level. The experimental treatments consisted generally in applying the ABDM combined with FA, in different concentrations. In order to compare the efficacy, a group of colonies were treated with oxalic acid (OA) by sublimation.

For brood decapping, a decapping fork was used (Fig. 1) the same used for honeycomb decapping, in order to superficially scrape the capping, without affecting the brood, opening it to increase to the maximum treatment effectiveness. One can mention that one full capped brood face of comb can be decapped in maximum 30 s, the specific movements in order to avoid destroying the brood can be watched in online presentation films [30, 31]. According to the older researches [27], the bees will recap the brood artificially decapped in the next period (12-24 h), as can be seen in Figs. 2 and 3, depending on different factors as brood age or colony strength.

After decapping, the stretched larvae get out of the cells because of their rotation movements in the cocoon formation process (Fig. 4). This stage is the most critical one in the decapping process. The “decapped” phase is a vulnerable period for varroa as some mites, in different stages, can escape out of cells affecting their reproduction success.

The experiments were carried out on 10 honeybee

Fig. 1 Artificial decapping method using a honey decapping fork.

Fig. 2 The brood recapping process after 30 min.

Fig. 3 The brood recapping process after 30 min (detail).

Fig. 4 This larval stage can be affected by decapping process as the larvae can fall down in the pupation process.

colonies in 2017 and 17 honeybee colonies in 2018.

The experiments performed in 2017 focused on the efficacy of the combined method on honeybee colony as a whole, while the experiments performed in 2018 focused only on the infested brood frames treatment, put in special boxes.

Regarding the treatment, the used liquid FA was between 60% and 65% concentrations during the experiments done in 2017 and between 65% and 85% concentrations during the 2018 experiments (Tables 1 and 2). The quantity of FA used in experiments in 2017 was between 25 mL/box and 60 mL/box and between 10 mL/box and 150 mL/box in the 2018 experiments. The FA was impregnated in special

Table 1 The various designed treatments for the evaluation of the formic acid (FA) effectiveness on varroa found in artificial decapped brood in the honeybee colonies, in 2017 experiments.

Item	Treatments 2017	FA administration		Treatment period (h)	Night temperature (°C)	Evaporated quantity/treatment period (g)
		Concentration (%)	Quantity (mL)			
1	T1 (FA-60-60-14)	60	60	14	8	8
2	T2.1 (FA-60-50-15)	60	50	15	15	31
3	T2.2 (FA-60-50-15)	60	50	15	15	31
4	T2.3 (FA-60-50-15)	60	50	15	15	31
5	T2.4 (FA-60-50-15)	60	50	15	15	39
6	T2.5 (FA-60-50-15)	60	50	15	15	39
7	T3.1 (FA-65-30-36)	65	30	36	12	15
8	T3.2 (FA-65-30-36)	65	30	36	12	22
9	T4.1 (FA-65-25-36)	65	25	36	12	11
10	T4.2 (FA-65-25-36)	65	25	36	12	11

Table 2 The various designed treatments for the evaluation of the FA and oxalic acid (OA) effectiveness on varroa found in artificial decapped brood put in special boxes, without bees, in 2018 experiments.

Item	Treatments 2018	Administration		Treatment period (min)	Time to maintain the frame in honeybee colonies after treatment until evaluation (h)
		Concentration (%)	Quantity (mL)		
I	FA, 65%, 100-150 mL, 15-35 min, normal evaporation (NE)				
1	T1.1 (FA-65-NE-100-15)	65	100	15	18
2	T1.2 (FA-65-NE-100-35)	65	100	35	48
3	T2.1 (FA-65-NE-150-25)	65	150	25	20
4	T2.2 (FA-65-NE-150-25)	65	150	25	70
5	T2.3 (FA-65-NE-150-15)	65	150	15	43
6	T2.4 (FA-65-NE-150-20)	65	150	20	42
7	T2.5 (FA-65-NE-150-20)	65	150	20	70
II	FA, 65%, 10-22 mL, 15-22 min, rapid evaporation (RE)				
1	T3.1 (FA-65-RE-10-15)	65	10	15	40
2	T3.2 (FA-65-RE-10-15)	65	10	20	16
3	T3.3 (FA-65-RE-10-20)	65	10	20	16
4	T4.1 (FA-65-RE-15-15)	65	15	15	12
5	T4.2 (FA-65-RE-15-15)	65	15	15	36
6	T5 (FA-65-RE-22-22)	65	22	22	12
III	FA, 85%, 100 mL, 15-20 min, NE				
1	T6.1 (FA-85-NE-100-15)	85	100	15	24
2	T6.2 (FA-85-NE-100-15)	85	100	15	24
3	T6.3 (FA-85-NE-100-20)	85	100	20	24
4	T6.4 (FA-85-NE-100-20)	85	100	20	48
IV	OA, 2 g, 15 min, sublimation				
1	T7.1 (OA-2-S-15)	2 g		15	48
2	T7.2 (OA-2-S-15)	2 g		15	72
3	T7.3 (OA-2-S-15)	2 g		15	72

T1-T7.3 = different variants regarding the used substance, concentration, quantity, treatment duration, type of evaporation, temperature, or time for brood maintaining after treatment.

cardboards (150 mm × 170 mm × 4 mm) and cotton textile.

The exposure time to FA was 14, 15 and 36 h, respectively, in the 2017 experiments, using special impregnated cardboard pads for normal evaporation (NE), and between 15-35 min in 2018, using specific textile sheets. The different variants had as goal to identify the most suitable treatment for further researches.

The treatment in special boxes used both NE and rapid evaporation (RE), the latter being performed by using a source of heating (with electric resistance and controlled temperature by a thermostat) (Figs. 5 and 6).

Besides this, in 2018 there were used also three honeybee colonies treated with OA by sublimation, 2 g/colony. The FA used for the experiments in honeybee colonies in 2017, was impregnated in special absorbent cardboards, put on the top of frames in the honeybee colonies, after brood decapping method application.

For the experiments made in 2018, the FA was impregnated in an absorbent special cotton textile (towels machine type) put on the top and bottom of frames, in boxes for six Dadant frames. After decapping and treatment, the brood was reintroduced in the origin honeybee colonies to be recapped, the hives being equipped with bottom boards and control sheets. In all the experiments the decapped brood surface was between 50%-75% from a Dadant frame.

The measurements were focused on establishing the total dead varroa in different developmental stages out of the total number of varroa individuals found in brood, in conformity with the protocol applied in the screening for low varroa mite reproduction and recapping in European honey bees [32]. Moreover, the number of fallen varroa on the bottom board was registered, using the standard counting methods [33]. The death percentage allowed establishing the effect of FA on different stages of varroa (reproductive and phoretic mites) found in experimental units.

Fig. 5 The application of cardboards impregnated with formic acid (FA) on the nest frames (2017).

Fig. 6 The air-tight special box for treatment of artificially decapped brood with FA (2018).

3. Results and Discussion

3.1 The Experiments Performed in 2017, on Decapped Brood, Maintained in Honeybee Colonies Treated with FA

The results for the experiments performed on treated brood and honeybee colonies (2017, Table 3, Fig. 7) show that the most affected varroa stages were the protonymphs and deutonymphs, where in the 90% of the treatments, the protonymphs mortality percentages were over 80%, while in more than 70% of treatments the deutonymphs mortality percentages were over 80%.

One can notice that the best results were obtained in the T2.1, T2.2, T2.3 and T2.5 treatments, in which, for almost all varroa stages, mortalities of over 80% were registered.

The varroa counting was done on 23.4 infested cells on average/treatment, but the exact data for each treatment are shown in Table 3, column two.

The fallen varroa on the bottom board/treatment was recorded in column eight of Table 3, the average in the whole experiment being 237 mites. The number of fallen varroa was registered for the entire colony and the decapping surface (1 decapped frame/hive).

Generally, all the treatments were efficient in terms of protonymphs and deutonymphs mortality, which is a very good effect in decreasing substantially the level of infestation, but the optimum efficacy is reached when the FA is used in the amount of 50 mL of 60% concentration for 15 h at 15 °C, the treatment being done in the evening and overnight when the temperature is more stable.

The recapping behavior gives sufficient time (12-24 h) for treatment to act inside. The effect of the treatment is less predictable in the case of mother

Table 3 The efficacy of different FA treatments combined with artificially brood decapping method (ABDM) on varroa mortality measured on all the developmental stages, in decapped brood, treated in honeybee colonies (2017).

Item	Treatments 2017	Number of counted infested cells	Mortality (%) Out of total found mites in specific development stages					Fallen varroa on the bottom board/treatment (No.)
			Foundress	Male	Protonymph	Deutonymph	Daughter	
0	1	2	3	4	5	6	7	8
1	T1 (FA-60-60-14)	25	45.0	75.0	100.0	100.0	16.7	27
2	T2.1 (FA-60-50-15)	17	100.0	100.0	100.0	100.0	100.0	267
3	T2.2 (FA-60-50-15)	42	81.8	100.0	90.9	100.0	100.0	220
4	T2.3 (FA-60-50-15)	30	80.0	100.0	100.0	91.7	Not found	332
5	T2.4 (FA-60-50-15)	28	16.7	20.0	82.6	75.0	40.0	346
6	T2.5 (FA-60-50-15)	11	57.1	100.0	100.0	100.0	100.0	213
The average values for treatments FA-60-50-15		25.6	67.12	84	94.7	93.34	85	275.6
7	T3.1 (FA-65-30-36)	27	5.3	0.0	83.3	71.4	Not found	47
8	T3.2 (FA-65-30-36)	14	31.0	40.0	100.0	94.4	50.0	203
The average values for treatments FA-65-30-36		34	18.15	20	91.65	82.9	50	148.5
9	T4.1 (FA-65-25-36)	23	10.3	0.0	45.5	60.4	0.0	234
10	T4.2 (FA-65-25-36)	17	27.9	8.3	100.0	95.5	26.3	481
The average values for treatments FA-65-25-36		20	19.1	4.15	72.75	77.95	13.15	357.5
11	The average values of the whole experiment	23.4	45.5	54.3	90.2	88.8	54.1	237

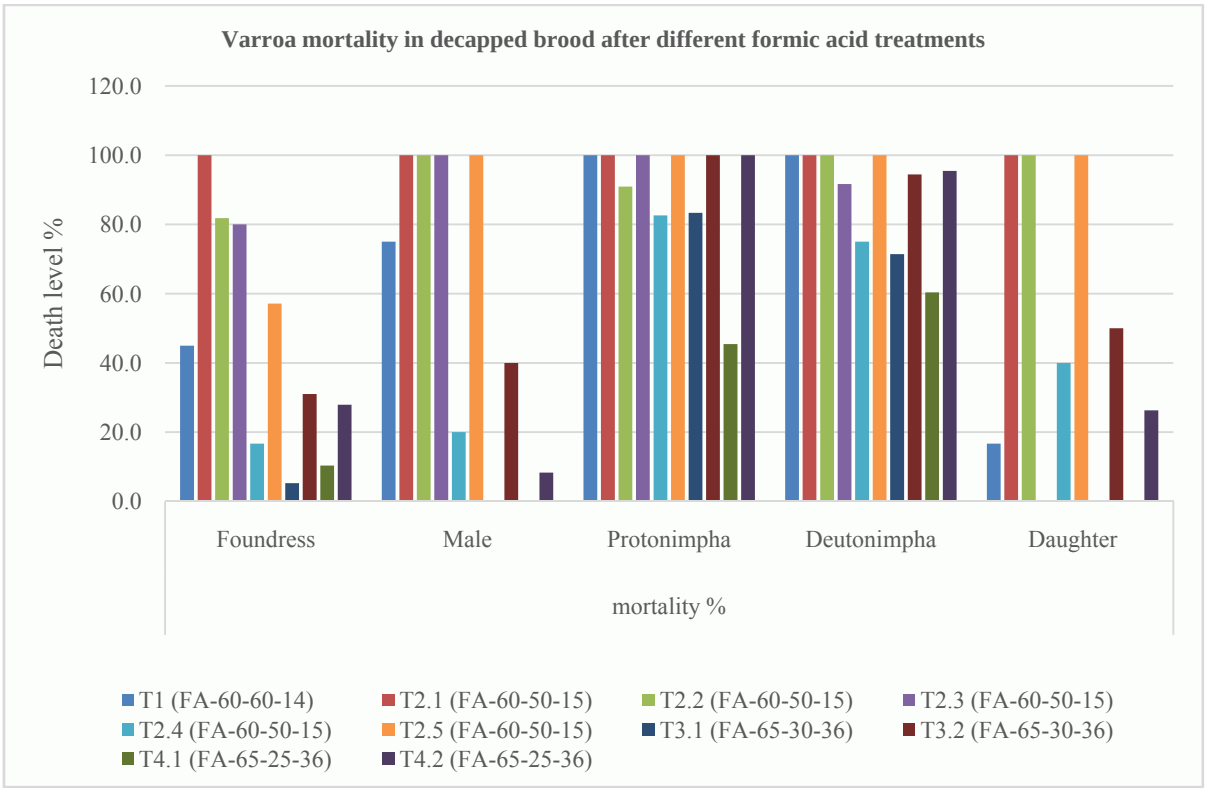

Fig. 7 The efficacy of different FA treatments combined with artificially brood decapping method (ABDM) on varroa mortality measured on all the developmental stages, in decapped brood, treated in honeybee colonies (2017).

foundress and daughter stages, where there were registered more variable data, these stages showing a better resistance to some treatments.

Thus, the mortality percentages varied between 10.3% and 100% in the case of foundresses, with an average of 45.5% and between 0 and 100% in the case of varroa daughters, with an average of 54.1%, depending probably on different factors, the most important being how well they are exposed to the substance and the level of substance concentration in different places of the cell or frames. The same situation is found in varroa males, too, where their mortality percentage was registered between 0 and 100% with an average of 54.3%.

In terms of mean values of different sets of treatments, the results show that the best treatment was the T2 (FA-60-50-15)—FA 60%, 50 mL applied for 15 h at 15 °C, the best effectiveness being registered on male, protonymph and deutonymph stages.

3.2 The Experiments Performed in 2018, on Decapped Brood, Maintained in Special Boxes without Bees, Treated with FA and OA

The results of the experiments performed on treated brood in special boxes, without bees (2018, Table 4, Figs. 8-11) show that the most FA affected varroa stages were the same as in previous experiment—the protonymphs and deutonymphs, where in 80% of treatments, the protonymphs mortality percentages were over 80%, while in more than 60% of treatments the deutonymphs mortality percentages were over 80%.

In Figs. 8-11, one can notice that the best results were obtained in the cases of T2.1 and T2.2 treatments for the 1st category of treatments (FA 65%, 100-150 mL, 15-35 min, NE), in the cases of T3.1 and T3.2 for the 2nd category of treatments (FA 65%, 10-22 mL, 15-22 min, RE), in the cases of T6.2, T6.3 and T6.4 for the 3rd category of treatments (FA 85%, 100 mL, 15-20 min, NE), and in the case of T7.2 for the 4th

Table 4 The efficacy of different FA and OA treatments combined with ABDM on varroa mortality measured on all the developmental stages, in decapped brood, treated in special boxes, without adult bees (2018).

Item	Treatments 2018	Number of counted infested cells	Mortality (%) Out of total mites found in specific development stages					Fallen varroa on the bottom board/treatment (No.)
			Foundress	Male	Protonymph	Deutonymph	Daughter	
0	1	2	3	4	5	6	7	8
I	1st category of treatments—FA 65%, 100-150 mL, 15-35 min, NE							
1	T1.1 (FA-65-NE-100-15)	10	0.0	0.0	57.1	68.4	0.0	27
2	T1.2 (FA-65-NE-100-35)	35	63.4	73.3	100.0	96.0	12.5	442
3	T2.1 (FA-65-NE-150-25)	16	37.5	80.0	84.6	94.4	100.0	93
4	T2.2 (FA-65-NE-150-25)	16	94.1	75.0	100.0	100.0	Not found	93
5	T2.3 (FA-65-NE-150-15)	22	5.0	33.3	88.9	83.3	0.0	36
6	T2.4 (FA-65-NE-150-20)	30	69.0	64.3	97.7	93.3	50.0	214
7	T2.5 (FA-65-NE-150-20)	16	26.7	0.0	78.6	77.8	75.0	214
8	Average values	20.7	42.2	46.6	86.7	87.6	39.6	159.8
II	2nd category of treatments—FA 65%, 10-22 mL, 15-22 min, RE							
1	T3.1 (FA-65-RE-10-15)	15	8.3	75.0	90.9	88.9	100.0	31
2	T3.2 (FA-65-RE-10-15)	30	11.5	60.0	91.3	100.0	71.4	142
3	T3.3 (FA-65-RE-10-20)	20	0.0	28.6	95.7	94.7	33.3	142
4	T4.1 (FA-65-RE-15-15)	35	5.9	16.7	62.2	68.8	10.0	164
5	T4.2 (FA-65-RE-15-15)	20	0.0	18.2	85.7	78.9	0.0	135
6	T5 (FA-65-RE-22-22)	20	10.5	0.0	88.6	60.6	66.7	72
7	Average values	23.3	6.0	33.1	85.7	82.0	46.9	114.3
III	3rd category of treatments—FA 85%, 100 mL, 15-20 min, NE							
1	T6.1 (FA-85-NE-100-15)	20	36.4	75.0	86.7	66.7	50.0	144
2	T6.2 (FA-85-NE-100-15)	6	20.0	100.0	100.0	100.0	100.0	46
3	T6.3 (FA-85-NE-100-20)	17	69.2	85.7	92.9	88.9	66.7	34
4	T6.4 (FA-85-NE-100-20)	15	90.9	83.3	100.0	100.0	0.0	83
5	Average values	14.5	54.1	86.0	94.9	88.9	54.2	76.75
6	Total average values on FA treatments	20.1	32.3	51.1	88.3	85.9	43.3	124.2
IV	4th category of treatments—OA 2 g, 15 min, sublimation							
1	T7.1 (OA-2-S-15)	25	0.0	30.0	75.0	57.1	0.0	86
2	T7.2 (OA-2-S-15)	13	0.0	28.6	100.0	88.2	50.0	86
3	T7.3 (OA-2-S-15)	10	11.1	20.0	100.0	41.7	0.0	86
4	Average values on OA treatments	16	3.7	26.2	91.7	62.3	16.7	86

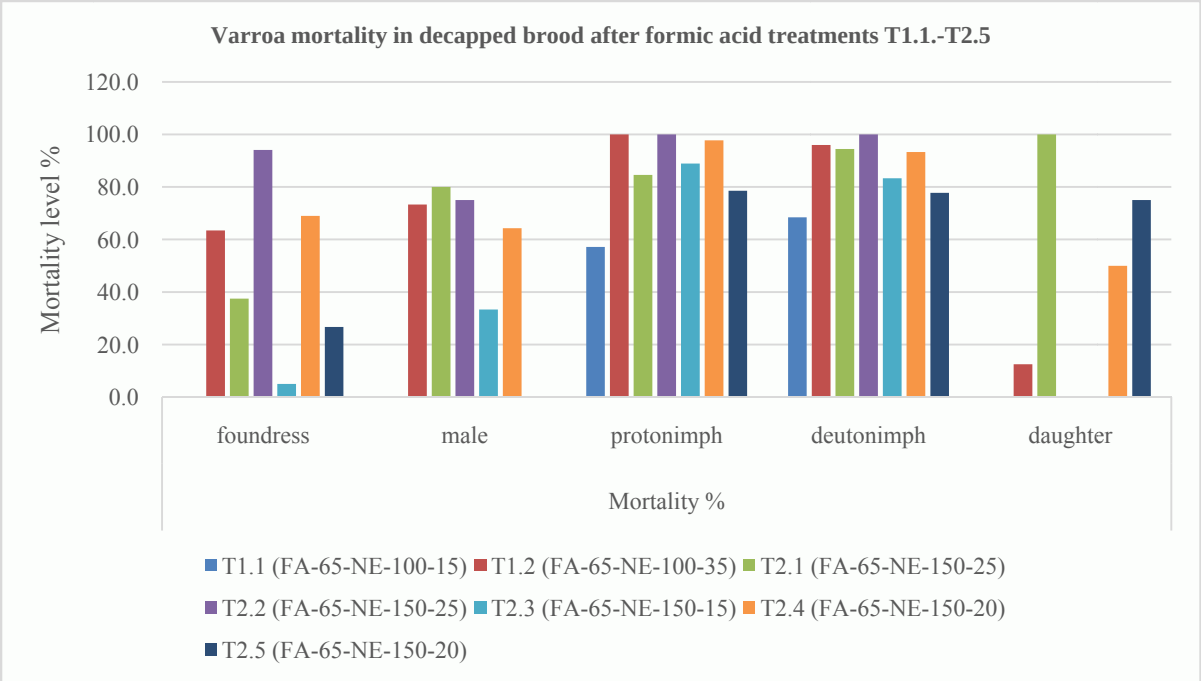

Fig. 8 The efficacy of different FA treatments combined with ABDM on varroa mortality measured on all the developmental stages, in decapped brood, treated in special boxes, without adult bees (2018), 1st category of treatments from Table 4.

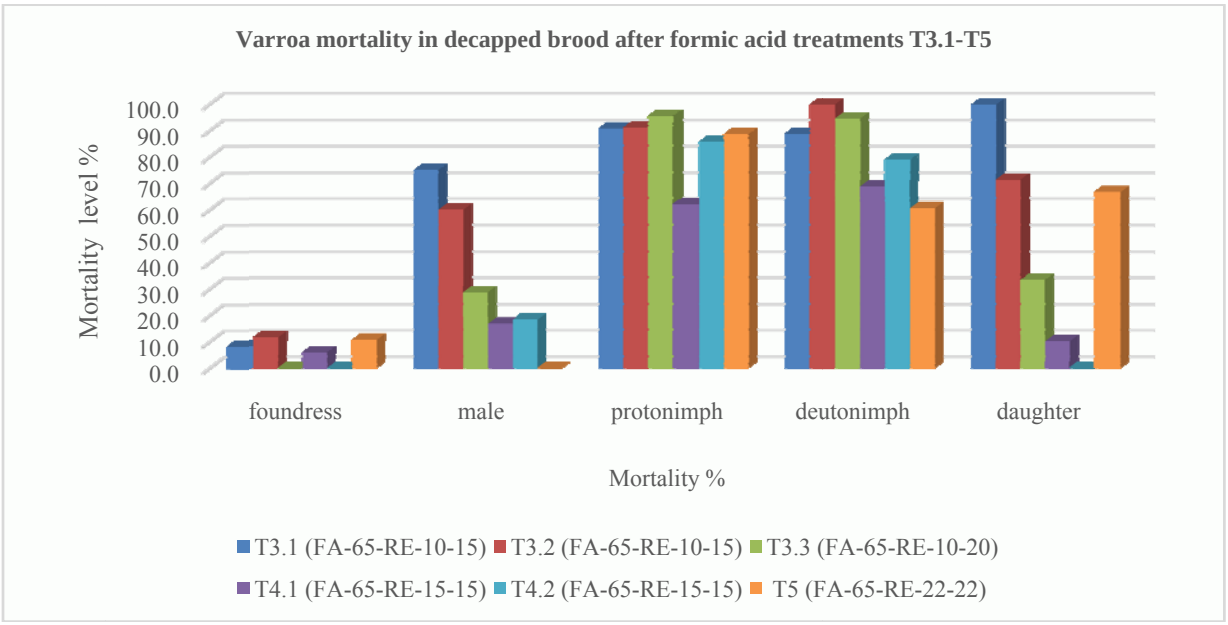

Fig. 9 The efficacy of different FA treatments combined with ABDM on varroa mortality measured on all the developmental stages, in decapped brood, treated in special boxes, without adult bees (2018), 2nd category of treatments from Table 4.

category of treatments (OA 2 g, 15 min, sublimation), where in almost all varroa stages mortalities of over 70% were registered.

The varroa counting was done on 20.1 infested cells on average/FA treatment and 16 infested cells on average/OA treatment, but the exact data for each treatment are shown in Table 4, column two.

The fallen varroa number on the bottom board/treatment was recorded in column eight of Table 4, the average of the whole experiment being 124.2 mites/FA treatment and 86 mites/OA treatment.

Fig. 10 The efficacy of different FA treatments combined with ABDM on varroa mortality measured on all the developmental stages, in decapped brood, treated in special boxes, without adult bees (2018), 3rd category of treatments from Table 4.

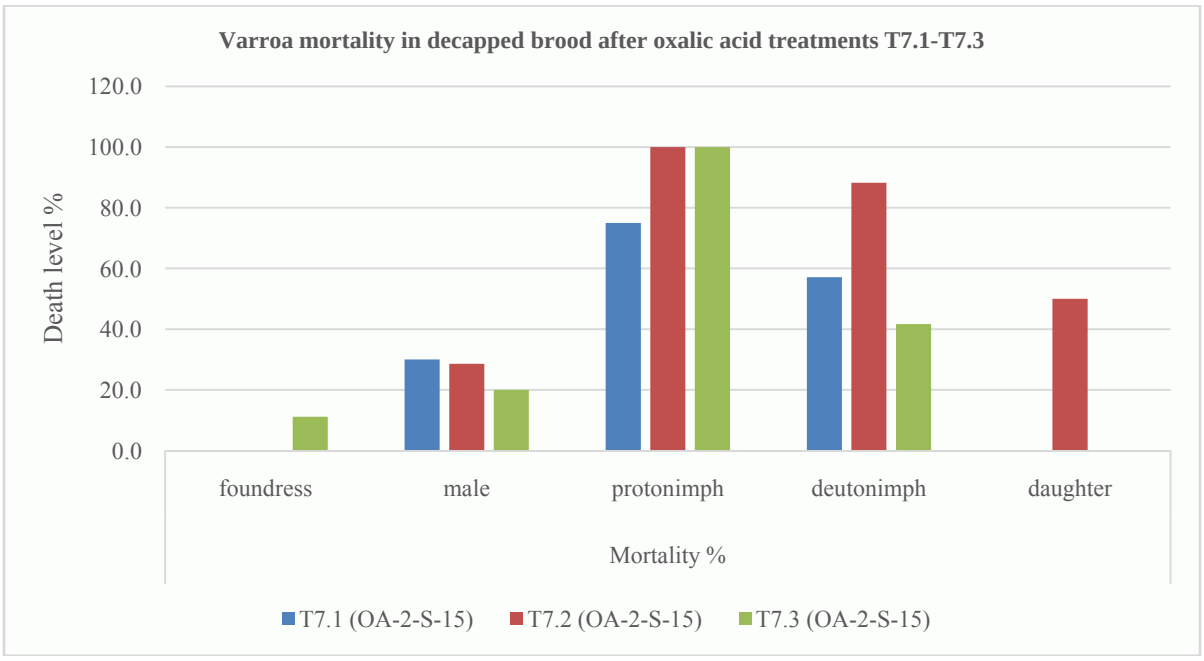

Fig. 11 The efficacy of different FA treatments combined with ABDM on varroa mortality measured on all the developmental stages, in decapped brood, treated in special boxes, without adult bees (2018), 4th category of treatments from Table 4.

Generally, as in the previous experiments, all the treatments were effective in terms of protonymphs and deutonymphs mortality.

The results in 2018 experiments show that, for the first category of treatment with FA of 65% concentration in NE, to treat the decapped brood isolated in special boxes, the optimum efficacy was reached when the FA was used in quantity of 150

mL/box/treatment for 25 min.

Regarding the second category of treatments that focused on lowering the quantity, as well as the time of treatment, by using a rapid method of evaporation, the results show that a quantity of only 10 mL/box/treatment is sufficient to have a good efficacy in only 15 min of treatment.

Regarding the third category of treatments that focused on a higher concentration of FA (85%), by NE, it was noticed that a good efficacy in terms of varroa mortality in decapped brood was reached in 15-20 min of treatment, by using a smaller quantity of FA (i.e., 100 mL), as compared with the first category of treatments.

In the case of OA treatment, a lower efficacy was obtained as compared to FA, when an amount of 2 g of OA was used by sublimation, keeping the decapped frames of brood for 15 min in the closed box.

All these treatments in boxes, as they involve a short application time, can be done at any moment of the day.

A special mention should be done on the effect of FA on the decapped brood. In all the experiments the recapping process done by honey bees was normal as in the non-treated colonies [27] and the honeybee colonies performed normally after the treatments.

4. Conclusions

The most protonympha and deutonympha in the cells are killed by FA after decapping treatment in both experiments—2017/2018, which shows that these treatments interrupt the life cycle of varroa in its reproduction phase.

The other stages (male, mother, foundress, daughter) can suffer, but the variation of mortalities is relatively large (from 0 to 100%), the variations being caused probably by how well they are exposed to FA and the degree of exoskeleton chitinization, these stages being less susceptible to treatment.

Analyzing the treatments done on brood in honeybee colonies it was noticed that the effectiveness of FA is higher in the upper side of the frames with brood, probably because of the ventilation done by honeybees.

Generally, the brood is much more resistant to FA vapors compared with the emerging bees and adult bees. The damages of brood could appear in the following situations:

(1) The freshly capped larvae in the pupation process can be damaged by decapping;
(2) The FA vapors affect the emerging brood and sometimes young larvae.

Following these experiments, some practical recommendation regarding the optimization of treatment with FA would be the use:

(1) In honeybee colonies, in combination with ABDM, using a quantity of 50 mL of 60% concentration for 15 h at 15 °C, in the evening and overnight when the temperature is more stable in the summer days;
(2) In brood boxes, in combination with ABDM, treating only brood combs, without honeybees:
    (a) by natural evaporation using a quantity of FA of 65%-85%, between 100-150 mL, for 20-25 min;
    (b) by RE using a quantity of FA of 65%, between 10-20 mL, for 15-25 min.

All these treatment variants shorten the period of treatment, allowing the interruption of the life cycle of varroa in any moment of the active season and reproductive period, so reducing the varroa level infestation.

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