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ORIGINAL RESEARCH ARTICLE

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Acaricide efficacy and honey bee toxicity of three new formic acid-based products to control *Varroa destructor*

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The replacement of synthetic acaricides with organic treatments is a big challenge in modern beekeeping. In summer 2015, we carried out in Central Italy a study to compare the varroacide efficacy and the toxicity on honey bees of the formic acid evaporator Nassenheider Professional® with two new formic acid-based gel formulations: MAQS® and Varterminator®. In the same apiary with 32 colonies, four homogeneous groups with eight hives each were prepared. The mean acaricide efficacy of Nassenheider Professional® was 73.2 ± 12.5%, while MAQS® and Varterminator® showed a mean efficacy of 49.3 ± 14.9 and 81.2 ± 16.0%, respectively. Colonies showed a statistically significant reduction of adult bees only after the MAQS® treatment with respect to the control group. All the treatments showed absent or quite moderate queen mortality (MAQS® and in Varterminator® groups 12.5%, Nassenheider professional®, and control groups 0%). Our study indicates that Nassenheider Professional® and Varterminator® may represent a sustainable and effective solution to control mite infestation during summer in temperate areas.

Keywords: Nassenheider professional®; MAQS®; Varterminator®; Varroa; formic acid

Introduction

Varroa destructor (Anderson & Trueman, 2000) is the parasite responsible for most colony losses worldwide (Dietemann et al., 2013; Martin et al., 2012; Nazzi et al., 2012; VanEngelsdorp, Hayes, Underwood, Caron, & Pettis, 2011) and its control is still considered one of the most difficult aspects to manage for beekeepers. Ensuring adequate acaricide efficacy treatments to control mite infestation is a pre-requisite for healthy honey bees and a proper hive product yield.

By inhibiting the respiratory functions and acidifying the body fluids of mites (Bolli, Bogdanov, Imdorf, & Fluri, 1993) formic acid is effective on both phoretic (on bees) and reproductive (in the cells) phases (Rosenkranz, Aumeier, & Ziegelmann, 2010) and it is regularly used by beekeepers since the '80 s (Ritter & Ruttner, 1980).

Formic acid dispensers are the most common ways to carry out liquid formic acid treatments in the hives. The development of new strategies to mitigate the evaporation is fundamental to weaken the negative effects of high environmental temperatures on the honey bees survival like mortality of the queen, depletion of the colony, swarming of the hive, and reduction in productivity (Bolli et al., 1993). In recent years, a new dispenser called Nassenheider Professional® has been developed, which is on the market since 2014.

Different studies (Eguaras, Del Hoyo, Palacio, Ruffinengo, & Bedascarrasbure, 2001; Eguaras et al., 2003; Feldlaufer, Pettis, Kochansky, & Shimanuki, 1997; Ramos, Otero-Colina, Sánchez-Arroyo, Santillán-Galicia, & Tecante, 2010; Satta et al., 2005) identified possible solutions able to minimize the risks of handling formic acid and reduce the evaporation rate by blending the liquid in a gel matrix. Two new disposable products in gel have been registered in recent years: MAQS® in Hawaii (2009), Canada (2011), US (2011), UK (2013), New Zealand (2014) and in 18 European countries; and Varterminator® in Italy (2015) (Giusti et al., 2017).

In this study, we compared the varroacide efficacy and toxicity on honey bees of the Nassenheider Professional® used with formic acid 60%, MAQS®, and Varterminator® as summer treatments in Central Italy (temperate area).

Materials and methods

In summer 2015 (from July to August), we compared in the same apiary the performances of three formic acid treatments to control *V. destructor*: Nassenheider Professional®, MAQS®, and Varterminator® (Figure 1). All treatments were administered according to the indications given by producers.

The apiary is located in Central Italy, Rome province (42°03′41.9″N 12°0.8′24.2″E, 219 m above sea level) in a temperate climate area.

Nassenheider Professional® (Joachim Weiland Werkzeugbau GmbH & Co. KG Zimmermannsgasse 2, 15366 Hoppegarten, Germany) is a formic acid

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Figure 1. Formic acid-based treatments tested: Nassenheider Professional® dispenser (left); MAQS® strips (centre); Varterminator® tablets (right).

dispenser to be placed on the top of the frames of the brood chamber, with temperature ranges from 8 °C to 35 °C. We tested this evaporator provided with 290 ml of formic acid 60%, provided with the larger U-wick size, for a 10-day treatment, according to label indications for Dadant–Blatt beehives.

MAQS® (Mite Away Quick Strips) (NOD Europe Ltd., Europe Branch Office, Grosse Hohl, 67354 Roemerberg, Germany) is a veterinary medicine in strips where formic acid is absorbed in a gel matrix. Each strip contains 68.2 g of formic acid. For its administration, the current label indicates to place two strips for 7 days on the top of the frames in the brood chamber or, during the nectar flow, under the queen excluder. As indicated in the leaflet of the product, on the application day the maximum outside daytime temperature should be between 10 °C and 29.4 °C, and hot temperatures (33.3 °C) during the first 3 days may cause excessive brood mortality and queen loss.

Varterminator® (IZO S.r.l., Via San Zeno 99/A, 25124 Brescia, Italy) is a veterinary medicine that consists in two gel tablets of 250 g each containing 90 g of formic acid to be applied for 10 days on the top of the frames in the brood chamber and replaced with two other tablets to leave in place for 10 days more. The indicated mean daytime temperature to obtain the best performances with Varterminator® range from 15 °C to 35 °C.

The experimental hives were grouped into four homogenous treatment groups according to colony strength and the mite infestation level. Each group was composed by eight fully populated Dadant–Blatt beehives: one group for each formic acid treatment, plus one untreated control group.

To evaluate the toxicity of the treatments at the beginning and end of each administration, the number of adult bees, sealed, and unsealed brood was estimated adopting the Liebefeld method (Delaplane et al., 2013). Visual estimations were carried out always at the same time of day in early morning before flying activity of bees (Delaplane et al., 2013). In order to calculate the acaricide efficacy and plot the mite fall dynamics, the mites killed by the above-mentioned formic acid treatments were counted every 2–3 days. At the end of each formic acid treatment, all dispensers, strips, and tablets were removed and the residual amount of liquid formic acid left in the dispensers was measured.

The protocols were devised according to the Varroa Control Task Force methodology of the COLOSS association (see Supplemental Material) and the EMA guideline on veterinary medicinal products controlling *V. destructor* parasitosis in bees (EMA, 2010). The mites killed by the treatments inside the brood cells were calculated by counting the mite fall for 12 days after the end of each formic acid treatment. At the beginning of the trial, the absence of drone brood was ensured.

The residual number of mites was evaluated with a follow-up treatment carried out with:

- two trickled oxalic acid solution (Apibioxal®, Chemicals Laif, spa, Vigonza, Padova, Italy) administrations, one at the beginning of a queen caging period of 21 days and one at the end of the queen caging period;
- a double dose (4 strips/hive) of Apistan® (tau-fluvalinate; Vita Europe Ltd, Basingstoke, Hants, United Kingdom), administered for all the queen caging period, until 7 days after the queen release.

The untreated colonies of the control group, used to verify the natural mite mortality, received the same above-mentioned follow-up treatments.

To calculate the acaricide efficacy (AE) obtained in each hive, the following formula was used:

$$\mathrm{AE} = \left(V_{\mathrm{F}}/V_{(\mathrm{F+FOLLOW\text{-}UP})}\right) * 100$$

where V_{F} is the total number of mites killed by each formic acid treatment and $V_{(\mathrm{F+FOLLOW\text{-}UP})}$ represents the total sum of the mites killed in the hive (including the follow-up treatments).

Mean, maximum and minimum environmental temperatures were recorded daily by Ibutton dataloggers (Maxim Integrated, San Jose, CA) that were placed inside an empty hive, located near the treated ones.

Statistical analysis was performed to verify if the acaricide efficacy and variations in adult bee populations, sealed and unsealed brood of treated groups were significantly different with respect to the control group.

Kruskal & Wallis Kruskal & Wallis,(1952) test was used to verify if colony strength and levels of natural mite fall prior to treatment applications were statistically similar. Mann and Whitney (1947) test was used to compare acaricide efficacy of treatment groups with respect control group. Kolmogorov–Smirnov test was used to check the normality of distributions and equality of variances within groups regarding colonies strength (before/after treatments) was verified with Fisher's *F*-test. Student's test was applied in case of assumed homoscedasticity. All tests were applied using XLSTAT™ software.

Results

The four experimental groups had comparable strength and levels of natural mites fall prior to treatment applications (Table 1).

All the 290 ml dose of formic acid in the Nassenheider professional® dispenser had evaporated by the 10th day of administration.

Nassenheider professional® and Varterminator® treatments resulted in significantly higher acaricide efficacy with respect to the control group (Figure 2; Table 2). Comparing the efficacies among treatment groups, the acaricide efficacy of the Nassenheider professional® (Mann–Whitney test $p = 0.013$) and Varterminator® (Mann–Whitney test $p = 0.011$) were significantly higher than for the MAQS® treatments, while no significance was found between Nassenheider professional® and Varterminator® (Mann–Whitney test $p = 0.183$).

It was possible to verify different trends in mite fall dynamics (Figure 3): a general increase in the slope of mite mortality for all the treated groups during the first 3 days of formic acid administration; after the 3rd day (7th of August) the percentage of mites fallen in the Nassenheider Professional® group overcame the MAQS® group; and after the 5th day (9th of August) it also overcame the Varterminator® group. However, later on the 24th day (28th of August), the Varterminator® mite fall overcame the Nassenheider Professional® final acaricide efficacy.

The percentages in adult bee population, sealed and unsealed brood coverage after the treatments, with respect to the beginning of the study are presented in Table 3. A significant reduction in number of adult bees was observed in MAQS® group after the treatment and with respect to the untreated group ($p = 0.017$), while the unsealed brood increased after the Varterminator® treatment ($p = 0.033$) (Table 3).

Figure 4 sums up and compares the acaricide efficacy with the survival of adult bees. Adult bee variation was chosen in the graph because it was the only statistically significant reduction observed respect the control group. The intersection of the axes of the Cartesian planes was defined according to the higher value of miticide efficacy obtained in the control group and the mean toxicity observed for adult honey bees in the same group. Trying to summarize the overall performances of the four groups in a single figure (as in Giacomelli, Pietropaoli, Carvelli, Iacoponi, & Formato, 2016), if we consider points dispersion, it is possible to discriminate three main groups: the control group, that is located in the left part of the Cartesian plane; the MAQS® group, located in the central/left part and Varterminator® and Nassenheider Professional® groups located in the right part (corresponding to a higher acaricide efficacy).

The number of dead queens at the end of the treatment was one in MAQS® and in Varterminator® groups (12.5%) and zero in Nassenheider professional® and control groups.

Concerning the temperature ranges observed at the apiary level, the minimum daily temperatures recorded in the apiary never exceeded the minimum temperature ranges indicated by the producers. The mean daytime temperatures indicated for the treatments with

Table 1. Descriptive statistics and about strength of the colonies and levels of natural mite fall prior to treatment applications.

	Minimum	Maximum	Mean	Std. deviation
Number of adult bees before treatment[a]				
Nassenheider Professional® group	2260.1	12815.0	8854.0	3325.4
MAQS® group	6873.5	11184.0	8687.0	1496.0
Varterminator® group	5359.0	13048.0	8198.3	2741.1
Control group	5102.7	10601.5	7550.7	1549.5
Number of brood cells before treatment[b]				
Nassenheider Professional® group	3539.1	15,511.8	9789.0	4308.6
MAQS® group	7906.5	13,252.8	10567.1	2124.3
Varterminator® group	8433.6	18,448.5	13640.1	4016.3
Control group	5007.5	18,975.6	13182.2	5035.8
Total number of mites fallen before treatment[c]				
Nassenheider Professional group	7429	379,167	137,628	141,411
MAQS group	11,000	143,000	57,204	42,756
Varterminator group	29,571	118,667	62,718	31,317
Control group	5429	181,000	73,539	67,603

[a]Kruskal–Wallis test: K (observed value) = 3.339; K (critical value) = 7.815; DF = 3; *p*-value (two-tailed) = 0.342.
[b]Kruskal–Wallis test: K (observed value) = 4.315; K (critical value) = 7.815; DF = 3; *p*-value (two-tailed) = 0.229.
[c]Kruskal–Wallis test: K (observed value) = 1.040; *K* (Critical value) = 7.815; DF = 3; *p*-value (two-tailed) = 0.791.

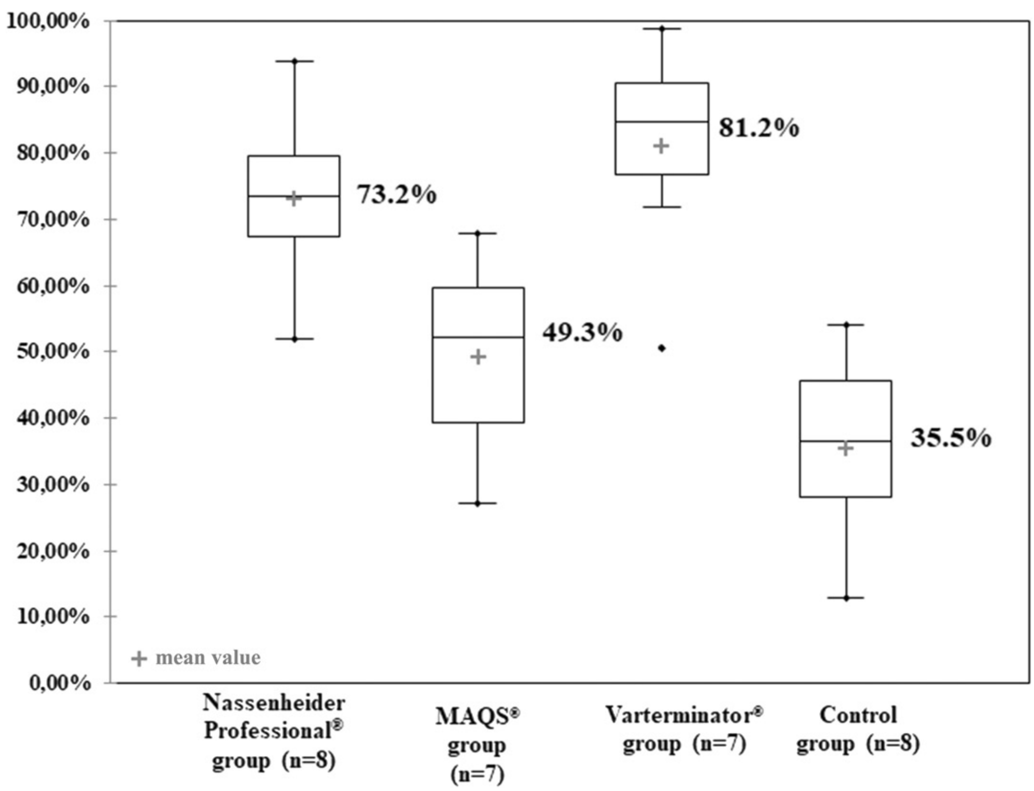

Figure 2. Acaricide efficacy boxplots.

Table 2. Descriptive analysis of acaricide efficacy.

Treatment group	Nassenheider Professional group ($n = 8$)	MAQS group ($n = 7$)	Varterminator ($n = 7$)	Control group ($n = 8$)
N	8	7[a]	7[a]	8
Minimum	52.0%	27.1%	50.5%	12.9%
Maximum	93.8%	67.7%	98.7%	54.0%
1st Quartile	67.5%	39.3%	76.6%	28.1%
Median	73.5%	52.2%	84.6%	36.5%
3rd Quartile	79.5%	59.7%	90.5%	45.6%
Mean (*p*-value**)	73.2% (0.001)	49.3% (0.118)	81.2% (0.002)	35.5%
Variance ($n - 1$)	1.6%	2.2%	2.5%	2.0%
Standard deviation ($n - 1$)	12.5%	14.9%	16.0%	14.3%

[a]The colonies where queen died after the treatment were not considered.
**Mann–Whitney test applied to acaricide efficacies of treated groups versus control group.

Nassenheider Professional® and Varterminator® were respected too. Part (5 of 10 days) of the Nassenheider Professional® and all (7 days) of the MAQS® treatment period was carried out when environmental temperatures were higher than those suggested by the manufacturers for the proper application of their products. More precisely, counting the number of hours through the Ibutton datalogger placed inside the empty hive near the treated hives, it was possible to assess that Nassenheider Professional® was carried out at off-range temperatures for 8.3% (20 of 240 h) of its treatment length, while MAQS® treatment was administered at off-range temperatures for 45.2% (76 of 168 h) of its treatment length. In contrast, Varterminator® was always applied respecting the suggested temperatures.

Discussion

V. destructor is considered the most important threat for beekeeping worldwide. Among organic compounds, beekeepers may use different products available on the market, but frequently it is not easy to figure out the most effective and at the same time least harmful treatment to adopt. The trial was performed after super removal, during the first half of August, which is the period of the year when usually acaricide treatments are carried out in Italy.

Concerning the temperature ranges observed at the apiary level, the deviation from the ranges specified in the respective labels for each treatment is a possible explanation for the statistically higher adult honey bee mortality observed in the MAQS® group

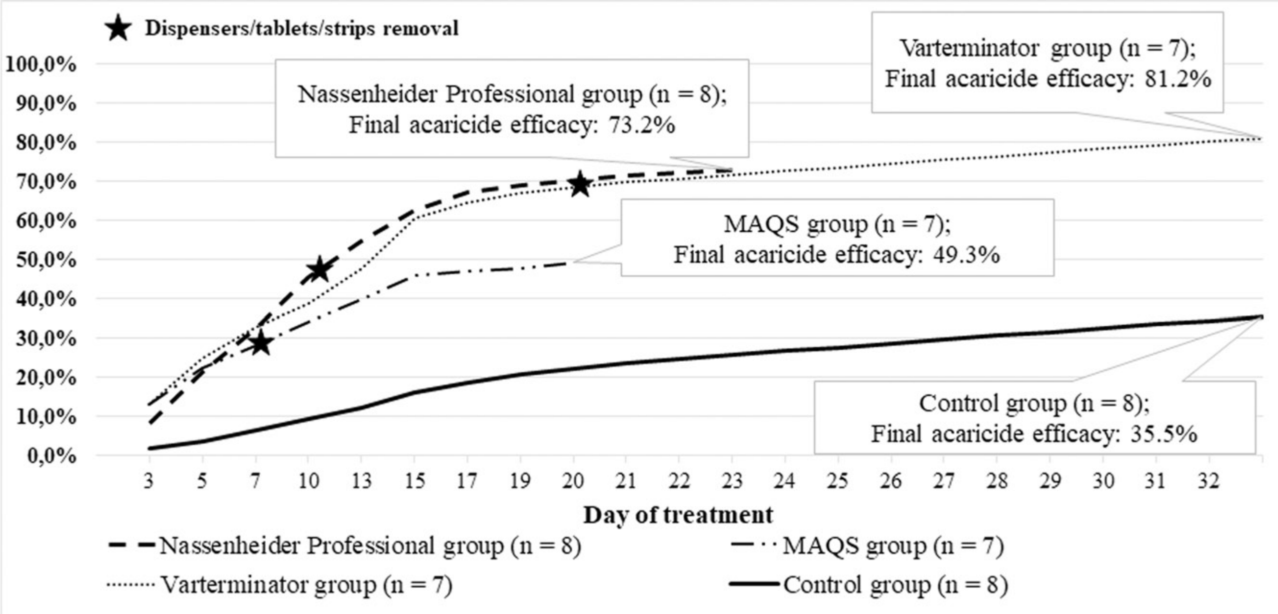

Figure 3. Mite fall dynamics obtained by counting mites every 2–3 days. Day 1 = 5th of August.

Table 3. Percentage of adult bees, sealed and unsealed brood after treatments with respect to the beginning of the study (100%) (mean ± S.E.) and *p*-values of tests.

Groups	Nassenheider Professional® group	MAQS® group	Varterminator® group
Adult bees	76.7 ± 24.4%	64.7 ± 10.3%	76.3 ± 21.6%
Fisher's *F*-test	0.589	**0.013**	0.416
T-test	0.145		0.110
Mann–Whitney test respect the control group	0.272	**0.017**	0.183
Sealed brood	181.4 ± 125.6%	136.0 ± 60.2%	51.5 ± 36.3%
Fisher's *F*-test	0.529	0.604	0.616
T-test	0.211	0.233	0.102
Mann–Whitney test respect the control group	0.118	0.478	0.073
Unsealed brood	163.6 ± 82.4%	154.5 ± 76.4%	199.6 ± 112.9%
Fisher's *F*-test	0.605	0.801	0.230
T-test	0.232	0.211	**0.033**
Mann–Whitney test respect the control group	0.862	0.747	0.685

(Table 3) that was lower in the two other groups as also suggested by Pietropaoli and Formato (2018) and Bolli et al. (1993). Even though tested products should be less dependent of climatic influence if used within the temperature range given by the producer, that derives from clinical trials, further trials should be conducted to better understand the influence of intra-colony and environmental parameters on efficiency of formic acid treatments for *V. destructor* control.

The highest acaricide efficacy (81.2%) was obtained by Varterminator® having a longer administration of formic acid (20 days long treatment) (Figure 3). Probably, an increase in the amount of formic acid in the Nassenheider Professional® dispenser, or a longer time in its administration (e.g. adopting the smaller U-wick size) could lead to an increase in the acaricide efficacy of this treatment, but further studies should be conducted to confirm this hypothesis.

A similar study performed in the same time of the year with liquid formic acid in Central Italy was carried out with different dispensers by Marinelli et al. (2007). Even though we found similar miticide efficacies, the most important differences in their study were the higher values in queen mortality, probably due to the higher dosages and concentration (85%) of formic acid they adopted.

In our trial, MAQS® showed a lower acaricide efficacy (49.3%) with respect to other previous studies (Giacomelli et al., 2012; Pietropaoli et al., 2011, 2012) we carried out in the same area, probably due to the out-of-range temperature conditions reached. Further studies should be carried out to verify if more interesting performances could be reached with MAQS® during spring or autumn seasons with lower environmental temperatures.

Finally, Varterminator® in our study showed a lower efficacy (81.2 ± 16.0%) with respect to other tests carried out in Italy in 2013 by Giusti et al. (2017) that found a mean efficacy equal to 94.62%. The absence of statistically significant differences in the mortality for

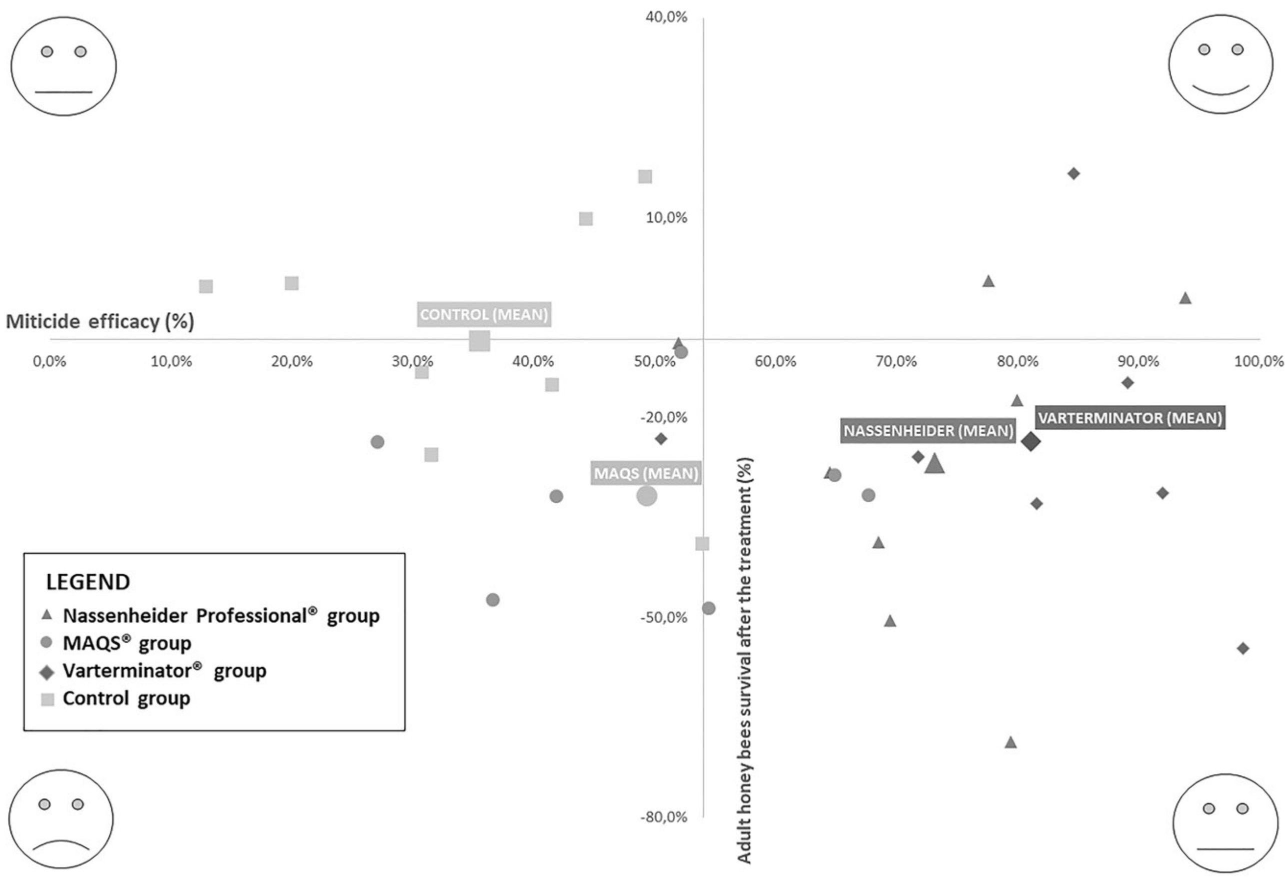

Figure 4. Plot of acaricide efficacy (*x*-axis) and percentage of adult honey bees respect the beginning of the treatment (*y*-axis) in the four groups. Legend: Nassenheider Professional® group (NASS); MAQS® group (MAQS); Varterminator® group (VART); untreated group (CONTR).

adult honey bees (queens included) after Varterminator® treatment reported by Giusti et al. (2017), indeed, were confirmed in our study. Moreover, a general increase of unsealed brood at the end of all treatments was observed and it was statistically significant in the Varterminator® group. This aspect seems to be linked to an increased egg-laying activity at the end of the formic acid treatment that could be related to the replacement of eggs and larvae that died at the beginning of the administration.

In general, considerable standard deviation in brood coverage was observed in all groups; further studies on the impact of formic acid on honey bee biology and population dynamics should be carried out, also considering the effects of long administrations.

Considering the user-friendly features and feasibility of the three treatments for beekeepers, Nassenheider Professional® evaporator entails the potentially dangerous management of liquid formic acid, the use of empty supers (these are not always available in summer after the honey harvest), to gain an additional volume to lodge the evaporator in the hive. On the other hand, MAQS® and Varterminator® have an advantage to be disposable products, even though Varterminator® too requires some extra-space to stock the product and the beekeeper is forced to go back to the apiary twice in order to remove the exhausted pads.

In conclusion, in our study, Varterminator® and Nassenheider Professional® showed the best performances in terms of acaricide efficacy and honey bee tolerability in our field conditions (temperate climate). At the meantime, MAQS® group showed the highest mortality among hives highlighting that compliance with the suggested environmental temperatures needs to be considered to choose the most appropriate treatment. Further studies are needed to improve data about performances of formic acid registered products and find the most appropriate solutions.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Supplementary material

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