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NOTES AND COMMENTS



Liquid and solid matrix formic acid treatment comparison against Varroa mites in honey bee colonies

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ABSTRACT

Beekeepers use organic acids to control the invasive parasitic mite *Varroa destructor* (Varroa mite) in honey bee colonies (*Apis mellifera*), as an alternative to synthetic acaricides, to which Varroa mites can develop resistance. Here, we tested the efficacy of two formic acid treatments for Varroa mite control: a solid matrix product (Formic Pro; $n = 10$ colonies) and a liquid product (Formivar 60; $n = 10$ colonies), relative to control colonies that were not treated with formic acid ($n = 10$). Both formic acid treatments killed $>95\%$ of the mites, however, the use of formic acid also resulted in 1.6 times more brood loss and 30% queen loss, relative to control colonies. Although the solid application was perceived as being more practical to use for treating against Varroa mites, both the solid and liquid application methods were equally effective and had similar negative side effects on honey bee colonies.

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The mite *Varroa destructor* Anderson & Trueman (2000) is an obligate parasite of honey bees *Apis mellifera*, L. It feeds on adult bees and pupae and vectors the deformed wing virus, which can lead to mortality of honey bee colonies (Genersch et al., 2010; Hasegawa et al., 2023). Therefore, managed honey bees require regular Varroa mite control. Organic acids such as oxalic acid and formic acid have become established treatment options in Central Europe (Brodschneider et al., 2023). Formic acid, unlike other treatment methods, penetrates through the porous surface of brood cell cappings and targets mites where they reproduce (Calis et al., 1998; Kubásek et al., 2022).

Though studies on formic acid show high efficacies in reducing mite infestation in honey bee colonies (Pietropaoli & Formato, 2019), beekeepers face the potential risk for skin and respiratory irritation when applying this treatment. Also, the use of formic acid requires careful consideration of dosage and application methods to prevent negative effects on honey bee survival and brood development (Ostermann & Currie, 2004). Since its efficacy can vary, e.g., based on the temperature, humidity and method of application, new application methods are being developed and marketed. We carried out a field trial to compare two formic acid medicines, registered for veterinary use in the Netherlands (REG NL 126198 and REG NL 118709, respectively), listed in an online database (Medicijnautoriteit CBG, 2023).

We note that only registered Varroa treatment products are permitted to be used in the Netherlands.

The Bijen@wur research apiary ($51^{\circ}59'32.6''\text{N}$; $5^{\circ}39'47.3''\text{E}$) was set up with 30 honey bee colonies: 10 colonies were treated with a solid matrix product (Formic Pro, NOD Apiary Products Ltd., Canada), 10 colonies received a 220 ml liquid application (Formivar 60, administered with a commercial “Liebig” dispenser, Andermatt Biovet GmbH, Switzerland), and 10 colonies were left untreated (negative control group). Throughout the study, all 30 colonies were fed sugar fondant since the colonies had little to no stored honey during the experiment.

The experiment ran from August 18th to October 18th, counting Varroa mites on bottom boards at two-day intervals. Treatments were applied on August 30th. The treatment duration was 36 days (Figure 1(A)). Two follow-up treatments were applied to kill remaining mites, by spraying the bees with a solution of 3.5% oxalic acid in water (October 4th and 10th). With queens caged three weeks prior, the follow up treatments were applied in absence of closed brood. Treatment efficacies were calculated as follows: $(\text{Number of mites killed by test compound} / (\text{Number of mites killed by test compound} + \text{Number of mites killed by the follow-up treatments})) \times 100$. To check impact on brood development, the number of frames in colonies that contained closed brood cells were counted before and after treatment (August

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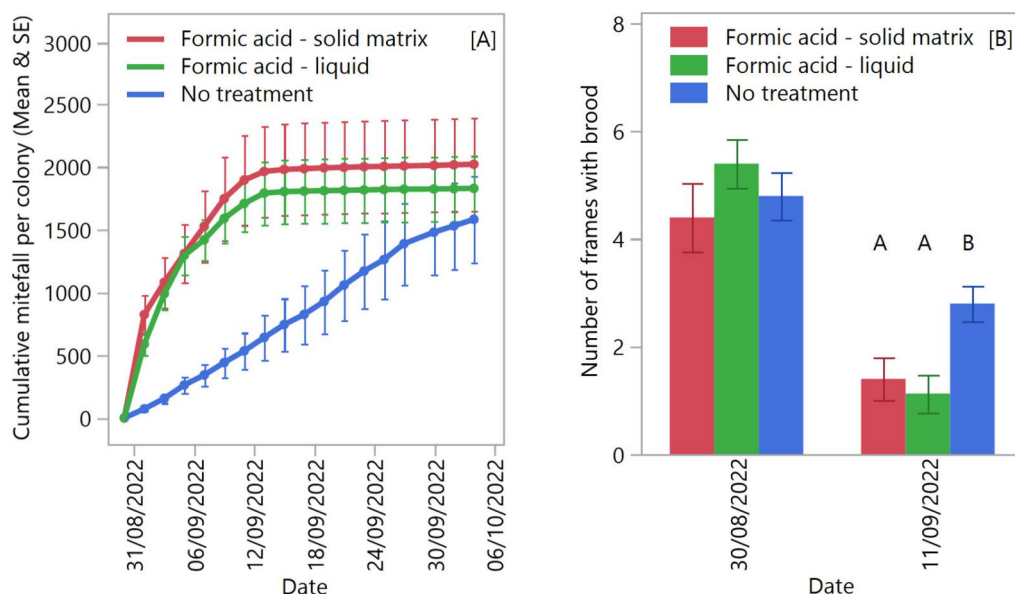


Figure 1. (A) Average cumulative mite counts of honey bee colonies. The formic acid treatments were applied August 30st–up to the follow treatments with oxalic acid (October 4th and 11th). (B) Number of brood frames in colonies, before and after treatment. Each treatment involved 10 replicate colonies.

30st and September 11th, resp.). Additionally, queen presence was verified by visual observation, before and after treatment (August 18th and September 11th). All collected data is accessible in an online repository (Hendriksma et al., 2023).

Between August 18th to October 18th 77,872 mites were counted, with mean 2596 mites per colony ($N=30$). Before treatment (12 days; August 18th to 30st) the mite fall did not differ between treatment groups (Kruskal-Wallis Test; $Df=2$, $ChiSquare=0.07$, $p=0.97$). The formic acid treated colonies groups showed an elevated mite-fall for 12 days (Figure 1(A)), i.e., the period that worker brood cells remain capped. The efficacy of killing Varroa mites was found to be $96.0\% \pm 1.0$ SE for the solid formic acid matrix and $95.4\% \pm 2.0$ SE for the liquid formic acid application method, with no significant difference between the two (one-way Anova: $F_{1,12}=0.06$; $p=0.81$). Multiple colonies were excluded because colony death or due to breeding drones.

Before the formic acid treatment, brood development did not differ significantly between treatment groups (one-way Anova: $F_{2,27}=0.94$; $p=0.40$; Figure 1(B)). Brood development in colonies did however declined significantly (one-way Anova: Figure 1(B); $F_{1,56}=60.9$; $p<0.001$). Relative to an average 2.00 frames brood loss in control colonies, the formic acid treated colonies lost 3.56 frames of brood (one-way Anova: $F_{2,25}=6.17$; $p=0.007$; Post hoc A A B; Figure 1(B)). Formic acid applications evoked notable stress in colonies, with five colonies exhibiting bearding outside the hive, and two colonies absconding. Queen loss in control colonies was 20% whereas in formic acid treatments the queen loss was approximately 50%, indicating a difference of

30% that may be attributed to the formic acid treatment, albeit as result not significant (Fisher's exact test, 2-tailed p -value = 0.13; $n=29$ observations), excluding one colony for analyses because the queen was killed by accident.

In practice, the solid treatment method was found easier in use (i.e., quicker and safer) than the liquid method. However, both methods effectively killed Varroa mites ($>95\%$). Despite the high efficacy, the results also highlighted several negative effects associated with formic acid use. In line with previous research our study revealed that formic acid treatment can result in increased worker bee and brood mortality (Ostermann & Currie, 2004), and induce queen supersedure. Therefore, to prevent adverse impacts on honey bee survival and queens, it is crucial to adhere to the appropriate dosage and follow the product label instructions (Van der Steen & Vejsnæs, 2021). Following instructions is essential to also prevent other negative impacts on colony health, beekeeper safety, and the quality of honey for consumers.

In adherence to guidelines, we treated one brood chamber with two Formic Pro strips. However, it is possible that higher concentrations than intended were reached, since the Dutch "simplex" hive type is 22% smaller in volume than the Langstroth hive for which the product was designed. The Liebig dispenser guideline regards colony size and hive type, and prescribed for our case to apply 2×150 ml Formivar 60: we however applied 1×220 ml. This dose evaporated with an average 22.7 ml per day in 12 days, being in the Liebig dispenser application target range of 20–25 ml per day.

The weather was 19.4°C and 69.2% relative humidity, on average. Notwithstanding, the daytime

temperatures often exceeded 25 °C, potentially escalating the formic acid evaporation. We further note extreme mite infections (Figure 1) with the colonies weakened by Varroa parasitosis (e.g., having Deformed Wing Virus). In all, our findings provide a worst-case representation of formic acid treatment practice and highlight associated negative consequences. While formic acid remains an effective treatment for Varroa in honey bee colonies, there is still room for treatment improvement regarding negative effects.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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