Efficacy of Api-Bioxal and ApiHerb against *Nosema ceranae* investigated by two q-PCR methods

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INTRODUCTION

Nosema ceranae parasitizes the honey bee ventriculum[1] and is transmitted by ingestion. This emergent pathogen may be vectorized by organisms other than honey bees[2,3] and is a major driver of *Apis mellifera* colony losses globally[4]. Control is problematic due to the limited availability of specific medicines.

Aim of the study: based on preliminary trials[5,6], **we compared the efficacy of the two environmentally sound products Api-Bioxal and ApiHerb against *N. ceranae*.** Quantification was made by q-PCR analysis based on two different gene sequences.

Figure 1. The products were dissolved in sugar water (1:1 w/v) and administered by trickling as per the label: once Api-Bioxal and three times (7 days apart) ApiHerb.

RESULTS

Abundance in pre- and post-treatment samples averaged $3.3*10^9$ and $4.8*10^5$ copies with the *Hsp70* method (Fig. 2). The second technique resulted in significantly higher values, respectively: $1.5*10^{12}$ (t-test: t(475) = -4.35; P = 0.000) and $2.6*10^7$ (t-test: t(475) = -5.08; P = 0.000) copies. Correlation was insufficient (r = 0.859, p=0.000) to make conversions between the two methods possible.

ANOVA showed a **significant treatment effect** on post-treatment data (F(2,16)=25.98, p=0.000). A post-hoc Newman-Keuls test detected significant differences for all pairwise comparisons (ApiHerb<Api-Bioxal<Control, α=0.05).

Figure 3 shows that, during the treatment, the abundance increased in the controls (+40.52%), but **decreased with Api-Bioxal (-98.75%) and ApiHerb (-99.98%)** (t-test: t(11) = -2.009; P = 0.070)). The decrease was of 1-6 orders of magnitude, the maximum value corresponding to the highly infected colony 3 of the Api-Bioxal group.

Prevalence was 100% in the pre-treatment samples of all groups. The parasite was still prevalent in all post-treatment samples from controls and Api-Bioxal treated colonies, but **a decrease was recorded in the group treated with ApiHerb**.

Three out of six ApiHerb treated colonies resulted negative at the post-treatment analysis with the *Hsp70* method. In the samples above, the prevalence was not significantly different when measured with the two q-PCR methods (*Hsp70*: 50.00%; *16S rRNA*: 54.67%; t-test: t(10) = -0.154; P = 0.881).

MATERIAL AND METHODS

Random groups of colonies were made from an infected apiary in Bologna, Italy. Starting on 6 October 2017, two of them were treated with Api-Bioxal, a.i. oxalic acid, or ApiHerb, containing garlic and cinnamon extracts (Fig. 1). The control group was left untreated.

On days 0 and 21, workers were sampled (N=25) from the external combs of each colony. Abundance and prevalence were assessed by individual q-PCR analysis, with primers and probes designed on sequences of the *Hsp70*[7] and *16S rRNA*[8] genes.

Parametric statistics were calculated on transformed abundance data: $x' = \log(x+1)$.

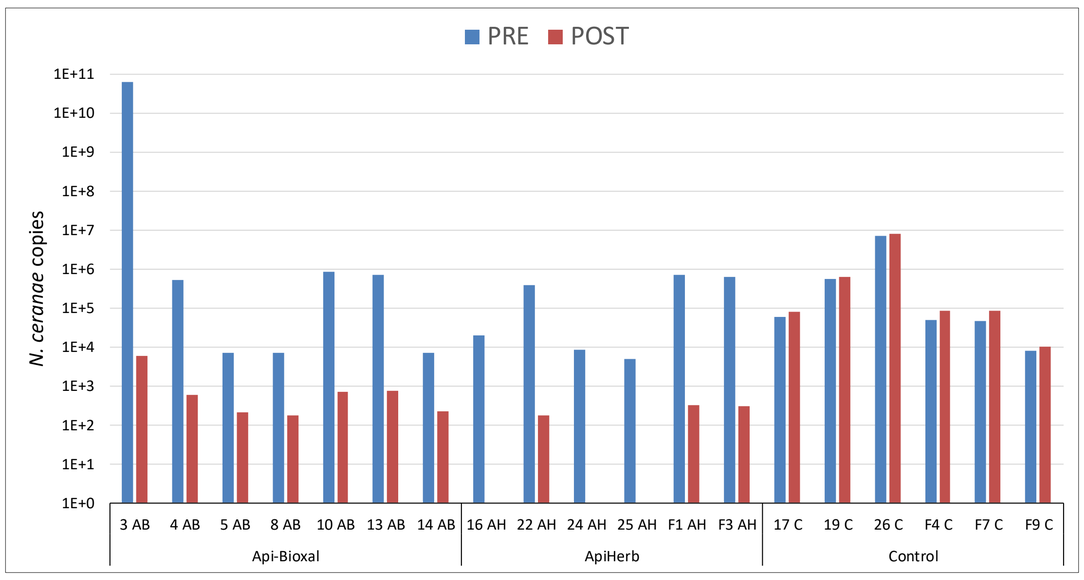

Figure 2. Average pre- and post-treatment abundance assessed by the *Hsp70* q-PCR method in the colonies of the three groups are shown pairwise.

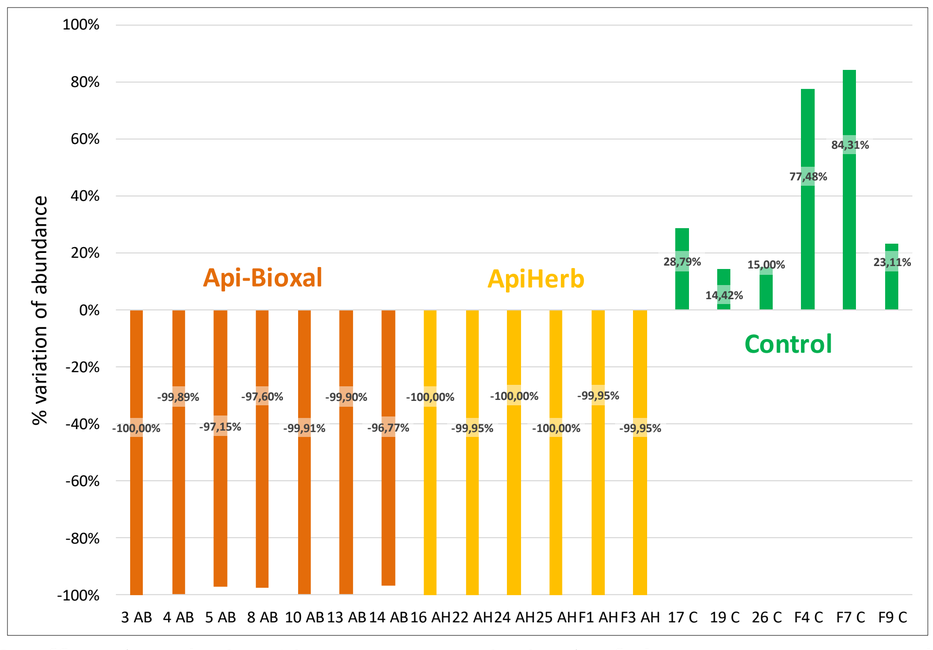

Figure 3. Effect (= relative % pre-post variation) of the treatments on abundance.

DISCUSSION AND CONCLUSIONS

Api-Bioxal reduced the abundance of *N. ceranae* copies but not the prevalence, unlike previous results[5] obtained with a distinct treatment protocol, i.e. two administrations of low concentrated oxalic acid solutions. However, our results suggest that Api-Bioxal treatments against varroa may produce an effect on coexistent *N. ceranae* infections also.

In this respect, a difference between Api-Bioxal and ApiHerb is weakly supported by the statistics. However, **ApiHerb affected the prevalence also**, although further experiments are needed to elucidate whether this depends on its specific composition or the extended treatment period.

Both products suit the needs of sustainable apiculture, and this experiment confirms that ***N. ceranae* can be controlled with environmentally safe substances**, at least in the conditions of this trial. However, research should be done to increase our understanding of their mode of action.

The ***Hsp70* and *16S rRNA* methods produced systematically different results**. Further investigation is required to clarify the underlying reasons and establish which technique is more reliable in quantitative analysis.

ACKNOWLEDGMENTS: The authors thank Dr Ilaria Cardaio and Dr Elisa Gianessi for their valuable technical support.

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