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European Journal of Integrative Medicine xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

European Journal of Integrative Medicine

journal homepage: www.elsevier.com/eujim

Research paper

Phosphorus 30 CH to control *Varroa* population in *Apis mellifera* colonies

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ARTICLE INFO

Article history:
Received 27 November 2015
Received in revised form 12 June 2016
Accepted 13 June 2016
Available online xxx

Keywords:
Phosphorus 30 CH
Varroa destructor
Homeopathy
Honeybee

ABSTRACT

Introduction: Varroa destructor is considered to be the main cause of European honeybee (*Apis mellifera* ssp. Linnaeus 1758) colony losses. The use of homeopathic products in veterinary practices has consistently increased in the last 50 years, but limited data are available on the application of homeopathic treatments to honeybees.
The aims of this study were to investigate the acaricide efficacy and tolerability for honeybees treated for 35 days with the homeopathic product Phosphorus 30 CH.
Methods: The clinical trial was carried out during the summer of 2012 in Central Italy. Twenty-four honeybee colonies were evenly divided into two different groups: one treated with Phosphorus 30 CH (12 colonies) and one left untreated (12 colonies). The mite mortality rate of the remedy was evaluated by counting the number of fallen mites on the sticky boards placed on the bottom tray of the hives every 3 days. Oxalic acid administration in an absence of brood was used to estimate the number of surviving mites. To assess the honeybee tolerability to the treatment, immediately before and after the Phosphorus 30 CH administration, an evaluation of the adult honeybee population was performed.
Results: The results revealed that no efficacy differences (p-value = 0.079, U = 23; EV = 45; Variance (U) = 150), nor differences in hive strengths (p-value = 0.118; U = 25.5; EV = 45; Variance (U) = 147.76) were observed in the treated group compared to the untreated group.
Conclusion: Our results are consistent with other studies conducted using homeopathic remedies to control varroa mites in *Apis mellifera* colonies. Further studies are needed to compare our data with different treatment durations, different administration methods and potency of the remedy.

1. Introduction

Homeopathy was developed in 1796 by Samuel Hahnemann. It is a system of alternative medicine based on the principle that *like cures like*, according to which, a substance causing the symptoms of a disease is able to cure the disease [9]. The use of homeopathic products in veterinary practices has consistently increased in the last 50 years [10,17].

Today, *Varroa destructor* (*V. destructor*), considered the main parasite of the European honeybee (*Apis mellifera* Linnaeus 1758), has spread to beehives worldwide, with the exception of Australia, and is a major threat for apiculture [21,8].

V. destructor is considered a crucial factor in the decreasing numbers of honeybee colonies and beekeepers [3]. Increased tolerance to different synthetic active molecules has been reported in strains of *V. destructor* that are resistant to fluvalinate, flumethrin and amitraz [1,5]. The use of natural treatments instead of hard chemicals and synthetic molecules has the advantage of limiting the drug resistance within the pathogens of farm animals [25,18,8], avoiding the environmental contamination and persistence of active compounds residues or their metabolites in the honeybee products. Moreover, homeopathic products can also decrease the cost of procedures and reduce health hazards to humans during treatment of animals [13,24]. Finally, the application of the homeopathic remedies should encourage beekeepers to adopt good farming methods [14], simultaneously enhancing the honeybees' welfare and immune systems [16].

Few data are available regarding the application of homeopathic treatments for honeybee diseases. In their preliminary studies, Sassoli et al. [23] tested the efficacy of *Calcarea Sulphurica* in improving the resistance to *Varroa* mites in beehives. Lotti and Martini [15] tested the efficiency of *Calcarea sulphurica* 200 CH and ApiBioxal® to improve the resistance to varroosis of honeybee

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http://dx.doi.org/10.1016/j.eujim.2016.06.007
1876-3820/

families treated in an absence of brood. With the same purpose, Persano and Marinelli [19] tested three homeopathic products: *Eureka, Apeas plus,* and *Apedin Vapor*. Other studies have been conducted by Ruiz Espinoza and Guerrero Salinas [22] that have attempted to build up an experimental protocol for the control of *V. destructor*, including the frequency of application and the potency (202 CH) of *Sulphur* and Varroa Nosode. To control *Varroa* mites in 2005, Flores [7] applied *Equisetum* spp. 60 CH with or without succussion directly to the feed administered to the honeybees.

Due to the lack of information on the application of homeopathy to honeybee diseases, this study aimed to investigate the tolerability of the *Phosphorus 30 CH* treatments on honeybees and its efficacy in improving resistance against *V. destructor*.

2. Methods

The field trial aimed to verify efficacy of *Phosphorus* 30 CH in improving resistance of honeybees to *V. destructor* after 35 days of treatment in presence of brood and to verify the honeybees' tolerability of this treatment. The clinical trial was carried out from June 2012 to the end of July 2012 in Rome province (Central Italy) on 24 honeybee colonies, located in the same apiary (41°43′28.1″N 12°42′57.1″E), housed in 10-frame Dadant-Blatt bee hives and free of any other symptomatic disease.

The 24 hives were divided into two homogeneous groups: (1) 12 colonies treated with *Phosphorus 30 CH* ("*Phosphorus*" group) and (2) 12 colonies left untreated ("Control" group). To evenly distribute the colonies in the two groups according to the Varroa population in each colony and the hive strength, the initial Varroa infestation level of the hives was evaluated by the natural mite fall counts recorded for two weeks before starting the trials [2], while the adult honeybee population was estimated by the Liebefeld estimation method [11].

The protocol is shown in Fig. 1 for the "*Phosphorous*" group and Fig. 2 for the "Control" group.

The *Phosphorus* 30 CH adopted remedy was selected according to scientific literature on symptoms transferred from humans to *Apis mellifera* ethological characteristics [4,12]. We considered as conceivable characteristics for honeybees: irritability; sensitivity (as reported by Kent [12]: "the patient phosphorus is very sensitive to all external sensations"); busy; perseverance; apprehensive during the "storm". One gram of *Phosphorus* 30 CH (Boiron laboratory Ltd, 20124 Milan, Italy) was diluted in 10 ml of water and the mixture was dissolved in a 1:1 ratio of sucrose, avoiding the use of metal tools to mix the product. Once a week, 1 ml of the *Phosphorus* 30 CH sugar solution was poured on a lump of sucrose positioned on the frames in the brood box (Fig. 3), for a total of 5 doses to cover 35 days of treatment.

To assess the honeybees' tolerability to the treatment, immediately before the first *Phosphorus 30 CH* administration (day 0) and one week after the 5th treatment (day 35), the adult honeybee population was evaluated using the Liebefeld estimation method [11]. Finally, the honeybee queens' vitality was recorded weekly during the 35 days of the treatment.

The experimental protocol (Figs. 1 and 2) in the apiary for testing the efficacy of *Phosphorus 30 CH* against *V. destructor* was conducted according to the EU guidelines [6]. For the field trial period, the mite fall was recorded every 3 days through the use of sticky boards put on the bottom tray of the hives.

To estimate the number of surviving mites after a period of 35 days of treatment administration, we counted Varroa fall produced by the administration of a single dose of trickled oxalic acid solution (Apibioxal, Chemicals Laif s.r.l.) after 24 days of queen caging with VAR-CONTROL® cages (Api-Mo.Bru, Campodoro, Padova, Italy – http://www.apimobru.com/en/ppe/ppe.htm) and considering a treatment efficacy of 90% [20].

The percentage of acaricide efficacy (AE) in each hive was evaluated using the formula:

$$AE = \frac{V_{\text{Phosph 30 CH}}}{V_{\text{Tot (Phosph 30 CH + Oxalic acid)}}} * 90$$

where V_{Phosph} is the total number of mites eliminated with the treatment with *Phosphorus* 30 CH, and $V_{Tot(Phosph30CH+Oxalicacid)}$ represents the total number of mites removed by the *Phosphorus* 30 CH treatment, plus the oxalic acid treatments.

The mean acaricide efficacy in the two groups ($\overline{AE}$) was evaluated using the formula:

$$\overline{AE} = \frac{\Sigma V_{\text{Phosph 30 CH}}}{\Sigma V_{\text{Tot (Phosph 30 CH+Oxalic acid)}}} * 90$$

Statistical analysis was performed to compare the efficacy of the treatment and the strength of the hives between the two experimental groups. The analysis only included colonies that had a level of infestation between 300 and 3,000 mites per colony, as indicated in the guideline on veterinary medicinal products controlling *V. destructor* parasitosis in bees [6]. Considering that the data do not come from a normally distributed population, the comparisons between groups were carried out with a nonparametric Mann-Whitney test for independent samples. The significance level was set to 0.05. All the statistical analyses were performed using XLSTAT software.

3. Results

The two groups, placed in the same site before starting the trial, presented homogeneous results, having a similar infestation level (p-value = 0.540; U = 37.0; EV = 45; Variance (U) = 150.00) and strength (p-value = 0.901; U = 47.0; EV = 45; Variance (U) = 145.395).

The acaricide efficacy observed in the "*Phosphorus*" group was 46.1%, while the acaricide efficacy observed in the "control" group

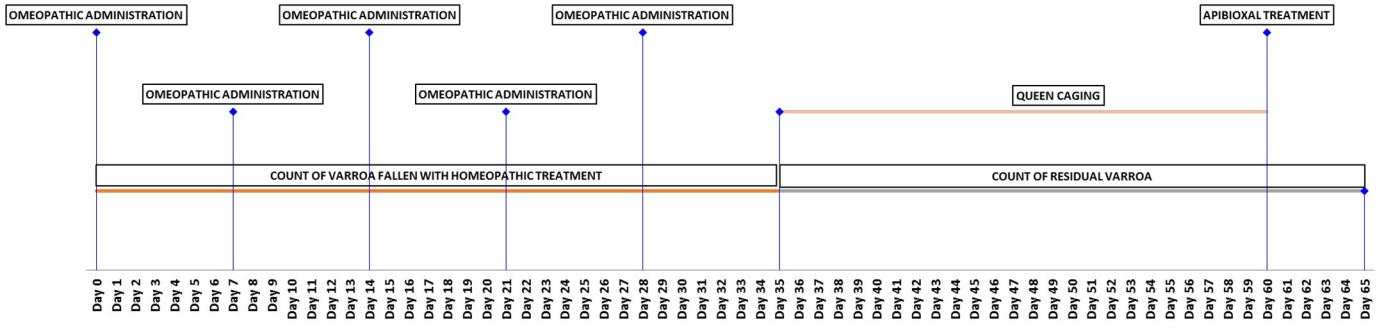

Fig. 1. Scheme of the protocol adopted for the "Phosphorus" group.

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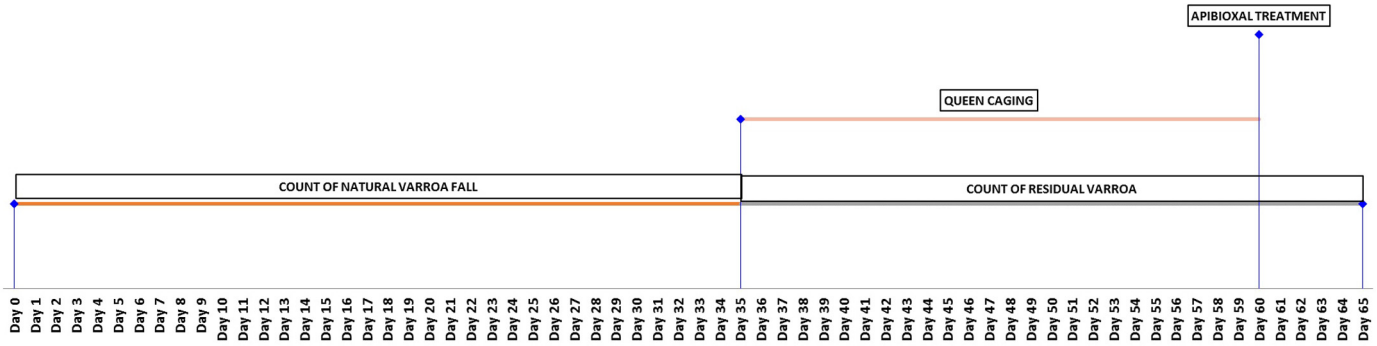

Fig. 2. Scheme of the protocol adopted for the "Control" group.

Fig. 3. Remedy administration to the honey bees by a lump of sugar.

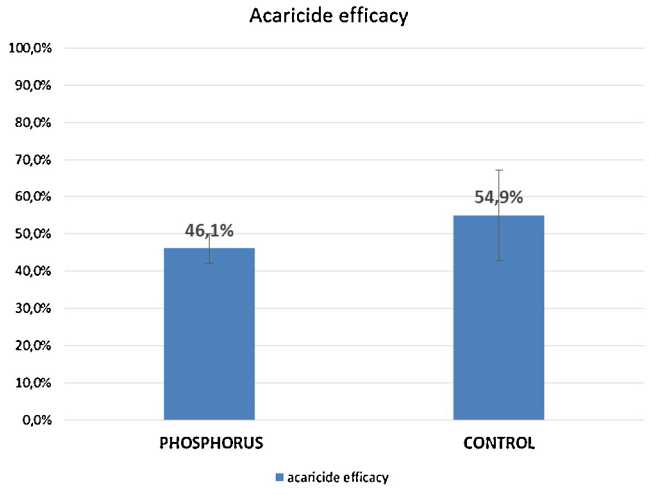

Fig. 4. Acaricide efficacy.

was 54.9% (Fig. 4). The standard deviation (SD) was 4.0% in the *Phosphorus* group and 12.1% in the Control group. No statistical differences were observed (p-value = 0.079, U = 23; EV = 45; Variance (U) = 150) between the two groups. Considering the toxicity of the treatment with Phosphorus 30 CH on the adult honeybees, in the "*Phosphorus*" group, after the treatment it was possible to observe an increase in the adult honeybee population of 19.74%, with an SD of 31.2%, comparing to an increase in the "control" group of 34.23% with an SD of 42.6% (Table 1). Even in this case, no statistical differences were observed between the two groups (p-value = 0.118; U = 25.5; EV = 45; Variance (U) = 147.76). After the treatment (day 35), the deaths of 3 queens in the "*Phosphorus*" group and 1 in the control group were observed.

4. Discussion

In our evaluation of the tolerability of the treatment, we did not observe any toxic effect on the adult honeybee populations, confirming the harmlessness of the homeopathic remedies.

However, we observed unsatisfactory results with *Phosphorus* 30 CH in the control of the *Varroa* population, perhaps due to the methods of use we adopted: duration of the treatment (35 days), method of administration (the lump of sugar once a week) and potency of the remedy (30 CH). Many factors that may interfere with the evaluation of the efficacy of homeopathic treatments must be considered, including the remedy, the potency of the remedy, the method of administration, the climatic conditions, the duration of the treatment and the honeybee subspecies [19,22,23]. Further studies modifying one or more of the factors mentioned above are needed to verify conceivable improvement in the control of the parasite.

It should also be taken into consideration that the homeopathic remedy must not have any acaricide effects, but aims to create a balance between host and parasite with infestation decrease.

Sassoli et al. [23] and Lotti and Martini [15] treated the honeybee families with *Calcarea sulphurica*, associating the treatment with Api-Bioxal®, and observed that the acaricide efficacy did not differ between the treated and untreated group. Persano and Marinelli [19] showed that after the treatments with the homeopathic remedies *Eureka* and *Apedin Vapor* (water, ethanol 19%, lactose and plant extracts: *Echinacea angustifolia*, *Thuya occidentalis*, *Spiraea ulmaria* and *Oxalis acetosella*)

Table 1
Evaluation of the strength of the honeybee hives in the two experimental groups before and after the *Phosphorus* 30 CH treatment.

	Pre-treatment (honeybees)	Post-treatment (honeybees)	Increase
"*Phosphorus*" group	51082.5	59190.59	54008.09 (+19.74%)
"Control" group	55136.44	70542.21	15405.77 (+34.23%)

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administered by dripping and *Apeas plus* (administered by spraying), the number of mites in the honeybee colonies persisted at high levels.

Ruiz Espinoza and Guerrero Salinas [22] tried to control *V. destructor* by the application of *Sulphur* 202 CH to the honeybees through impregnated sugar globules and noted no effective control of the mites. Finally, in 2005, Flores [7] found that *Equisetum* spp. 60 CH showed an efficacy of 57% in controlling varroa mites by direct administration to the feed of the honeybees. Our results are consistent with those presented in the above-mentioned studies.

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