

Experimental Treatment with the Natural Water Acidifier Provigoro® for *Nosema* spp. Control: Preliminary Results

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Abstract

Honey bee colonies in Peloponnesos, Greece, were sampled in spring 2014 to evaluate efficacy of Provigoro®, a natural water acidifier, against *Nosema* spp. Colonies treated with Provigoro® showed reduced *Nosema* spore load than did colonies that received only sugar syrup treatment, which finally collapsed. Preliminary results suggest that Provigoro® was successful at reducing nosemosis in *Apis mellifera* colonies.

Keywords

Honey Bee, *Apis mellifera*, *Nosema*, Nosemosis, Control, Provigoro

1. Introduction

Nosemosis of *Apis mellifera* is a bee disease caused by two different microsporidians: *Nosema apis* and *Nosema ceranae*. *Nosema ceranae* was first detected in European honey bees in 2006 [1] as a severe problem for beekeepers. Hatjina *et al.* [2] refer to *N. ceranae* as a significant factor of reduced honey production in Mediterranean countries. Infection occurs in adult midgut epithelial cells after spores are ingested during trophallaxis or cleaning of contaminated comb [3] [4]. As these effects can have a negative influence on professional beekeepers, it is important to develop strategies to control the disease associated with this pathogen. To control nosemosis, scientists recommend use of fumagillin bicyclohexylammonium [5]. However, fumagillin is not approved for apicultural use in EU countries (except Fumidil B in the UK, with fumagillin bicyclohexylamine salt as active

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ingredient), since its registration has been withdrawn [6].

Provigoro 14WA Bee Care[®] is a natural disinfectant based on organic acids (Phyto Innovative Products Ltd, Middlesbrough, UK). It is produced from 100% natural raw materials and its effectiveness relies on the sour orange extract and specifically the content of the bioflavonoids. The extracted bioflavonoids are combined with a number of natural acids (like ascorbic, citric, lactic, etc.) to produce the core active substances which have a proven broad spectrum efficacy against harmful micro-organisms.

Due to the need for alternative treatments, in the present study we evaluated the effectiveness of Provigoro 14WA Bee Care[®], against nosemosis.

2. Materials and Methods

The experiment was conducted during February and March of 2014. During the experiment, 6 honey bee colonies of the same apiary, heavily infected by *Nosema* spp., were randomly divided in two experimental groups, each containing three colonies. Colonies of the first group were treated with Provigoro[®] (1 ml in 1000 ml of sugar syrup in 1:1 dilution with water), while colonies of the second group served as control (treated with sugar syrup in 1:1 dilution with water). In each treatment, 100 ml of sugar syrup were sprayed per colony twice per week and a total of 10 treatments were applied. During the experiment, none of the colonies showed symptoms of other honey bee disease and none received any other treatment.

Samples of 5 forager bees collected from the entrance were collected from each hive before and at the end of the treatments. *Nosema* spp. infestation was calculated by spore counting, according to Cantwell [7]. According to this, abdomens of the bees were separated and grinded in a mortar with a pestle. In the mortar, 1 ml of water per bee was added and a drop was placed in a haemocytometer. Spores were counted under microscope in 400X magnification. Differences in mean values between groups were analyzed with the Mann-Whitney U non-parametric test for the comparison of the treatment and control samples.

3. Results and Discussion

Results showed that there was a significant reduction (Mann-Whitney, $p > 0.05$) in the number of spores per bee after the treatments with Provigoro[®] (Table 1). Comparison between treated colonies and the control was not able to be done, since control colonies were collapsed before the end of the experiment.

Greece is the country with the highest hive density in the world, with over 11 hives per km². It also has the largest population of professional beekeepers in the European Union. Any disturbance of colony health or reduction of annual honey yield reflects to the reduction of beekeepers income and, thus, in reduction of the apicultural share of national GDP. Microsporidan genus *Nosema* includes two of the most prevalent pathogens implicated in honey bee colony losses [1] [2] [8]-[12]. *Nosema ceranae* is the aetiological agent of an emerging illness now known as type C nosemosis [13]. *Nosema* spp. are known to affect both the foraging behaviour and life span of bees, increasing the likelihood that foragers may not return to the colony [8] [9] [13] [14]. Also, Botias *et al.* [15] referred that there is evidence for negative effects of colony parasitization by *Nosema* spp.—primarily by *N. ceranae*—on the effectiveness of acaricide strips to treat *Varroa destructor*.

Preliminary results of our study show that treatment with Provigoro[®] reduced infestation of *Nosema* spp. in the colonies. Considering the aforementioned high importance of nosemosis on beekeeping and since fumagillin is not registered for colony treatment against nosemosis in EU countries, treatment with Provigoro[®] would be an effective alternative. Also, since Provigoro[®] is registered as organic water acidifier, it is also compatible with organic beekeeping.

Further studies are needed to elucidate the mode of action, determine the optimum concentration and treatment replications before Provigoro[®] can be pursued as a tool for use in bee colonies disease management programs.

Table 1. *Nosema* spp. spore infection levels (in 10⁶ spores/bee) in control and Provigoro[®]-treated bees before and after treatment.

	Provigoro [®]	Control
Pre-Treatment	>15	>15
Post-Treatment	3.87 ± 3.71	N/A

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