Nozevit Aerosol Application for *Nosema ceranae* Disease Treatment

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Abstract

Recently, results of experimental *Nosema ceranae* and *apis* disease treatment with Nozevit were published. These results showed that a large number of nosema spores were significantly reduced using a common feeder for preventive treatment schedule (70.91%), and curative treatment schedule (78.37%) of honeybee colonies; and also after different methods of Nozevit application (drenching with sugar solution – 81.92% reduction; feeding with pollen patties – 96.70% reduction). The aim of this new research was to assess the effectiveness in the treatment of honeybee colonies affected with *Nosema ceranae* disease with an aerosol applicator of Nozevit phyto-pharmacological preparation, at manufacturer's recommended amount of product, but in a reduced sugar solution base (10 mL) as opposed to the industry standard drenching method (250 mL), thereby reducing cost per hive by saving beekeepers time and sugar solution.

Introduction

Honey bees are an extremely important part of natural ecosystems (Pham-Delegue et al. 2002). They represent the main factor of food chains (plants – animals – humans), they enhance agricultural productivity and help maintain biodiversity by engaging in pollination (Williams, 1994; Delaplane and Mayer, 2000; Morse and Calderon, 2000) and produce many apian products (Yue et al., 2006). The health and vigor of commercial honeybee colonies are threatened by numerous pathogens and can also be infested by several parasites. It is conceivable that these diseases have a huge effect on honeybee health, and consequently affect the profitability of apiculture and agriculture.

Among the bee diseases, nosema disease of adult honey bees is a serious one, caused by two described species of microsporidia, *Nosema apis* (Zander, 1909) and *Nosema ceranae* (Fries et al., 1996). Honeybee colonies are frequently infected, including all colony members. The disease occurs throughout the world and causes significant detriment to honey production that results in economic losses. *N. ceranae* is now a common infection of European honey bees and is highly pathogenic to its new host (Fries et al., 2006; Cox-Foster et al. 2007; Higes et al., 2007; Huang et al. 2007; Klee et al., 2007). The asymptomatic nature of *N. ceranae* disease means that controlling the condition is fraught with difficulty (Martin - Hernandez et al., 2007). Because the European Union, as well as Croatian regulations, prohibit the use of antibiotics, i.e., fumagillin, in the treatment of apian diseases, the need arises for the production and utilization of natural phyto-pharmacological preparations in the treatment of nosema disease. Nozevit is a natural preparation produced as a water solution of plant polyphenols, and is approved and registered in Europe as a "partner for nosema disease repression".

This experiment was designed to test the effectiveness of repeated treatments with Nozevit herbal preparation to control nosema disease in field conditions. Recently, results of experimental nosema disease treatment with Nozevit were published (Tlak Gajger et al., 2009a; Tlak Gajger et al., 2009b; Tlak Gajger et al., 2011). These results showed that a large number of nosema spores was significantly reduced using a common feeder with preventive (70.91%), and curative (78.37%) treatment of honeybee colonies; and also after different ways of Nozevit application (drenching with sugar solution – 81.92% reduction; feeding with pollen patties – 96.70% reduction).

Aerosols are liquid or solid particles that remain suspended in the air for an extended period of time. The particles remain suspended because they are very small and, therefore; do not fall rapidly under force of gravity. Just as liquid and solid aerosol particles can atomize in air, so can this sediment be dispersed on the surface of the honeybee's body. We tested the activity of Nozevit using an aerosol application method, by means of which each bee could receive a part of the herbal preparation on account of their social behavior. It involves taking of all dispersed sugar syrup solution and its "proboscis to proboscis" sharing where the active substance could be spread across the entire bee colony with minimal honeycomb storage. So, the aim of this part of our research was to assess the effectiveness in the treatment of honeybee colonies affected with *N. ceranae* disease by aerosol application of a Nozevit phyto-pharmacological preparation.

Materials and Methods

The field part of the experiment was conducted during 40 days beginning of July 20, 2010 in the apiary situated in the continental part of Croatia. Twenty-four hives were selected and before field testing 60 bees per colony were taken from the hive entrance and examined under microscope for the presence of nosema spores, and by molecular multiplex PCR for Nosema species determination.

After that, hives were divided into testing (18) and control (6) groups each having similar amounts of sealed brood area. Testing groups were simultaneously treated with Nozevit aerosol, using a specially designed aerosol "top" attached to a compressed air line which under compression produces aerosol. The treatment of the colonies was provided every ten days, three treatments in total. We used 1 mL of Nozevit suspended in 10 mL sugar solution per colony and application time was 30 seconds, respectively. Also, three different dilutions of the recommended rate of Nozevit were used (pure 100%, 50% water solution and 25% water solution). For control groups of hives, pure sugar solution was used, applied in the same way.

Each time before the next treatment we took 60 bees per colony from the hive entrance and examined them under a microscope for the presence of spores, and determined their number by counting in a haemocytometer according to Bürker – Türk (Cantwell, 1970). Samples were taken from about 60 adult bees at the hive

entrance before, and on the 10th, 20th and 30th day after first treatment. Bee samples were collected into clean plastic receptacles around 16 o'clock, and prepared for laboratory examinations the same way as described in Tlak Gajger et al. (2009b). We used 400x magnifications under a bright field microscope Olympus BX41 and took photographs with Olympus DP12 U-TVO camera (Photos 5 and 6). The counting equipment was carefully washed after each sample counting in order to avoid contamination with spores from the previous sample.

Determination of Nosema species was performed as in Tlak Gajger et al. (2009b).

Results

The results of microscopic examination of *N. ceranae* spore presence in honeybee samples after the field testing of Nozevit applicated by aerosol, before, and on 10th, 20th and 30th day after its introduction are provided in Table 1.

Results showed that a considerable reduction in spore numbers was achieved after first treatment (78.38%) with 100% pure Nozevit and 50% diluted Nozevit (54.34%). The other two treatment results were not as effective in spore reduction (50.08% after 2nd treatment and 36.59% after last treatment). All other results for dilutions of 50% and 25% of Nozevit were not effective, and the percentage of reduced spores was very similar with the control – non treating hive groups. The results of PCR amplification with generic *Nosema* primer pair perfectly matched the results of amplification with specific *N. ceranae* primer pair.

Discussion

Nosemosis is a parasitic disease of adult honey bees. Due to its inconspicuous signs, or asymptomatic duration, and the need for eradication by interchange of frames with brood in a disinfected hive, beekeepers devote insufficient attention or often neglect the disease. Also, because of official normative regulations, it appears to be necessary to introduce effective herbal preparations in treatment, which don't represent any possibility for accumulation of residues in honey and other apian products for human consumption. This experiment was designed to test the effectiveness of repeated treatments with Nozevit phyto-pharmacological preparation to control *Nosema ceranae* disease, in field conditions using experimental aerosol "top". Treatment was applied via aerosol method by using a special instrument "top" which under compression produces aerosol (Photos 1 – 4). It is ordinarily used for treatment with acaricides and other chemotherapeuticals, and has a wide variety of uses including applications against *Varroa destructor*. We assumed that a preliminary small-scale study would demonstrate whether Nozevit applied by aerosol has the potential for effective treatment of bee colonies suffering from nosema disease, and compare it with other, already tested and effective ways of application (Tlak Gajger et al., 2009a; Tlak Gajger et al., 2009b). Compared with previous testings and despite failure to achieve complete cure with any of the applicated ways of treatment with Nozevit, it needs to be stressed that treated groups of honeybee colonies had a significantly reduced number of spores compared to the control groups. Our results after aerosol application showed that a considerable reduction in spore numbers was achieved just after the first treatment (78.38%) with 100% pure Nozevit and 50% diluted Nozevit (54.34%), and the other two treatment results were not as effective in spore reducement (50.08% after 2nd treatment and 36.59% after the last treatment). So, we can conclude that Nozevit preparations (in sugar syrups applied in feeders or "drench" method and in pollen patties) works in field conditions (commercial beeyards), and ensure normal physiological processes in the honeybee body (Tlak Gajger et al., 2011), if they are applied precisely according to label instructions. But, in view of achieved results, we do not recommend using just the aerosol application of Nozevit, or diluting of the preparation beyond the manufacturer's recommendations.

Table 1. Spore counts (per 0.04 mm) on initial day and on 10th, 20th and 30th day after first the aerosol treatment with Nozevit.

Nozevit dilution →		100%	50%	25%	Control group (pure sugar solution)
		Mean spore counts (per 0.04 mm)			
Initial sampling	Mean	**44.65**	**47.90**	**30.18**	**57.60**
	Min	37.68	28.18	14.75	50.62
	Max	51.62	67.62	45.62	64.50
After 1st treatment	Mean	**3.50**	**26.03**	**30.18**	**24.00**
	Min	3.25	15.00	8.18	24.00
	Max	3.75	37.00	52.18	24.00
After 2nd treatment	Mean	**22.81**	**17.09**	**8.65**	**15.46**
	Min	21.62	9.06	7.81	7.87
	Max	24.00	25.12	9.50	23.06
After 3rd treatment	Mean	**16.34**	**42.28**	**53.03**	**48.43**
	Min	9.12	11.00	29.56	26.81
	Max	23.56	73.56	76.50	70.05

Conclusion

Implementation of Nozevit by aerosol application is recommended just for one – first treatment of honeybee colonies with a necessary combination with other ways of Nozevit treatment during the honeybee active season.

ACKNOWLEDGMENTS

The author expresses sincere gratitude to beekeeper Stjepan Žeželj and student Tomislav Tlak for technical assistance in field conditions.

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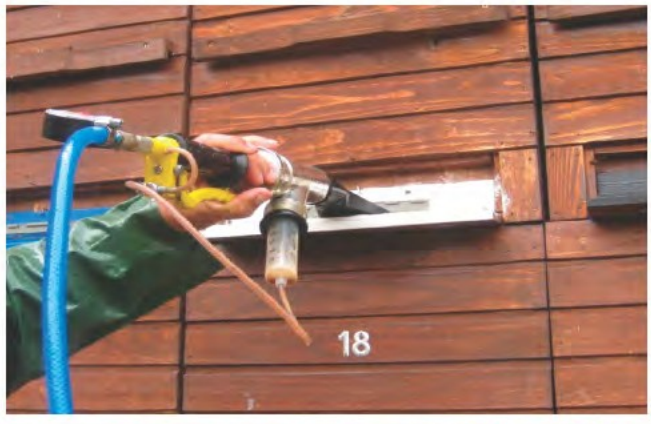
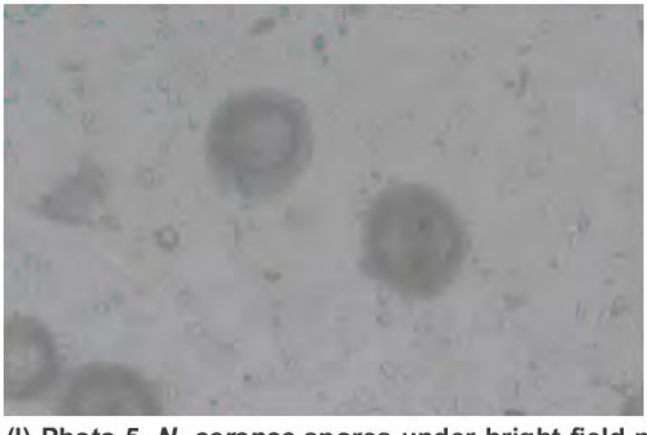
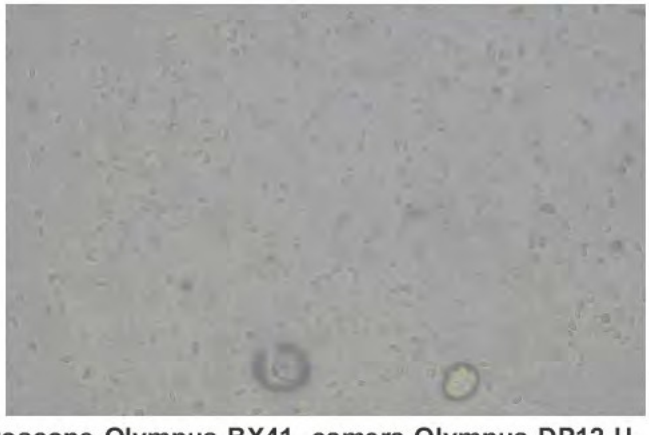



Photos 1 – 4. Using a special instrument "top", which under compression produces aerosol, for application of treating dose of sugar syrup with Nozevit.

(l) Photo 5. *N. ceranae* spores under bright field microscope Olympus BX41, camera Olympus DP12 U-TVO. (r) Photo 6. *N. ceranae* spores on the grid of the haemocytometer (according to Bürker – Türk) under bright field microscope Olympus BX41, camera Olympus DP12 U-TVO.

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