

## EVALUATION OF THE EFFECTIVENESS OF SELECTED TREATMENTS OF *NOSEMA* SPP. INFECTION BY THE HEMOCYTOMETRIC METHOD AND DUPLEX PCR

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Recent years have witnessed an increase in the mortality of honey bees in many regions of the world. The observed decrease in the bee population results from a combination of factors, and microsporidian parasites *Nosema apis* and *N. ceranae* are among the main contributors. Those parasites cause a microsporidian infection that shortens the lifespan of bees and reduces the productivity of bee colonies. The aim of this study was to evaluate the effectiveness of Nozevit, Api Herb and ApiX (acetylsalicylic acid + *Artemisia absinthium* L. extract) in the control of infections caused by *Nosema* spp. in a field experiment. Two groups of worker bees were evaluated – hive bees and forager bees returning to the hive. The effect of the analyzed therapies on the number of spores and the microsporidia species were analyzed by the hemocytometric method and duplex PCR. A statistical analysis revealed that the applied treatments had reduced the number of spores by 31.15% on average. In hive bees, *Nosema* spp. infection was most effectively reduced by Nozevit (67.85%) and ApiX (63.36%). Coinfections (*N. ceranae* and *N. apis*) were affirmed in all bee samples before treatments. However, after the treatments, single infection of *N. apis* and *N. ceranae* were detected. The tested treatments were more effective in the control of *N. apis* than *N. ceranae*.

**Key words:** Nosemosis control, *N. apis*, *N. ceranae*, honey bees

### INTRODUCTION

The global increase in honey bee mortality rates is associated with the widespread use of crop protection chemicals, monoculture farming and various diseases affecting bee colonies. The leading causes of adult bee diseases in honey bees are two microsporidian species of the genus *Nosema* (kingdom Fungi, class Microsporidia, family Nosematidae) – *Nosema apis* and *N. ceranae* along with *Varroa destructor* mite, honey bee viruses and their interactions [1-3]. These parasites cause microsporidiosis (nosemosis), an infection that spreads per os with contaminated food. The pathogens

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proliferate mainly in mid-intestinal epithelial cells [4]. In worker bees, microsporidian infections inhibit the development of hypopharyngeal glands that secrete royal jelly, thus disrupting the feeding of queens and the brood [5]. *N. apis* infections shorten the lifespan of worker bees and queen bees, leading to ovarian damage and infertility in queens [6]. The disease lowers honey production and can reduce brood by even 50%. *N. ceranae* infection develops rapidly and may be highly lethal. Bees die within 8 days after experimental exposure to *N. ceranae* under laboratory conditions [7]. However, in mixed infections (*N. apis* and *N. ceranae*), no competitive advantage was affirmed for *N. ceranae* for either infectivity or spore growth [8,9]. The symptoms of *N. ceranae* infections in bee colonies are visible throughout the year, whereas the disease spread by *N. apis* disappears in the warm months of the year. Symptoms traditionally as fecal marks, sick crawling bees attributed to *N. apis* infection were observed in the majority of *N. ceranae* infected bees [10]. In most cases, the disease spreads when healthy and infected colonies are merged and when contaminated hive equipment and food reserves are used. Young queen bees and worker bees from apiaries affected by noseiosis can also be a source of disease [11,12].

The main challenge facing beekeepers is to limit the spread of sporozoan infections in bee colonies. The use of the antibiotic Fumagillin DCH is not licensed in most countries of the European Union due to the side effects of its commercial formulation, like genotoxic and tumorigenic properties [13,14] and stability in honey [15]. The most popular alternative treatments against noseiosis including Api Herb, Nozevit, Feed Gold, Protofil and Nosestat, are not always effective [16-18].

The aim of this study was to evaluate the effectiveness of three preparations in the control of infections caused by *Nosema* spp. in a field experiment. Two preparations are already commercialized (Nozevit, and Api Herb), and the third ApiX (acetylsalicylic acid + wormwood *Artemisia absinthium* L., extract) is currently undergoing pre-clinical trials.

## MATERIALS AND METHODS

### ***Honey bee colonies, test treatments and experimental design***

In spring, the presence of *Nosema* spp. spores and *Nosema* species were determined in samples of winter hive debris from a commercial apiary, Poland (53°47'N, 20°30'E) (200 bee colonies kept in Dadant hives) by the microscopic method. To evaluate the effectiveness of the tested treatments an experimental apiary of 25 bee colonies was established. The outward symptoms of noseiosis were not observed in the analyzed colonies. All hives/groups were in similar health conditions before the experiment. The bees were divided into five groups. Group N was treated with herbal product Nozevit International® in sugar syrup (1:1 water/sugar) at 3ml/4l of syrup. Group AH had received herb-based preparation ApiHerb (Chemicals Laif srl, Italy) at 4g/50ml of syrup. Group AX administered ApiX (acetylsalicylic acid + extract of wormwood *Artemisia absinthium* L.), at 1.5mg/10l of syrup. Two control groups were included

in the study, one fed sugar syrup (Cs) and the other that was not additionally fed or medicated (C0). The treatments and sugar syrup were administered 4 times, every 2 days, in early April, at 0.5 l/colony in feeders that enable feed intake control.

The effect of the test treatments on *Nosema* spp. was analyzed by the hemocytometric method with the use of a modified Neubauer chamber and by duplex PCR. Sixty hive bees (HB) were randomly harvested from the brood comb in the center of the nest and 60 forager bees (FB) returning to the hive were randomly collected from the hive entrance using a special catching device. First samples were collected 7 days after the last administration of feed and test treatments. Overall, samples were taken 5 times, every 7 days around noon. Number of spores was determined in accordance with the guidelines of the Office International des Epizooties [19].

### **Duplex PCR**

To identify *Nosema* spp. all samples were analyzed using duplex PCR method and species – specific primers (321APIS-FOR/REV for detection of *N. apis* and 218MITOC-FOR/REV for detection of *N. ceranae*) designed by Martin-Hernandez *et al.* [20]. Genomic DNA was isolated from filtered and centrifuged (800 g for 6 minutes) sample suspensions using the Genomic DNA Mini Kit (A&A Biotechnology, Gdynia, Poland). Purified DNA was stored in test-tubes at a temperature of -20°C for further analysis. Duplex PCR reaction was carried out using HotStarTaq *Plus* Polymerase (Qiagen). The reaction mixture of 20 µl comprised around 120 ng isolated DNA (from 1 to 3 µl), 10µl HotStarTaq *Plus* Master Mix 2x, 2 µl CoralLoad Concentrate 10x, and 0.1 µl of each primer (with a final concentration of 0.5 µM). The reaction was carried out in the Mastercycler thermocycler (Eppendorf). PCR commenced with initial denaturation for 5 minutes at 95°C. The reaction mixture was then cycled 35 times, in the following steps: denaturation at 94°C for 45 seconds, primer annealing at 55°C for 45 seconds and extension at 72°C for 1 minute. The last reaction was followed by final chain synthesis at 72°C for 10 minutes. The products of the duplex PCR reaction were separated by electrophoresis in 2% agarose gel in 1x TAE at 5 V/cm. The size of the obtained products was evaluated by comparison with the GeneRuler 100 bp Plus DNA Ladder (Fermentas) molecular size marker. Ethidium bromide was added to the gel at 0.5 µg/ml to visualize the resulting DNA fragments of *Nosema* spp. Electrophoresis results were archived using the GelDoc (Bio-Rad) gel documentation system.

The results were processed in the Statistica 9.0 application for Windows using one-way ANOVA and Duncan's test ( $p \leq 0.05$  and  $p \leq 0.01$ ).

## **RESULTS**

Based on hemocytometric analysis of 25 bee colonies, the average number of *Nosema* spp. spores in HB from winter hive debris ranged from  $13.74 \times 10^4$  in group N to

25.62 x 10<sup>4</sup> in group Cs (Table 1). The average number of *Nosema* spp. spores in HB collected from the brood comb before treatment ranged from 18.36 x 10<sup>4</sup> in group AH to 28.0 x 10<sup>4</sup> in group N. A significant decrease in *Nosema* spp. spore counts was noted in HB from all groups as early as during the first analysis after 4 applications of Nozevit, Api Herb, ApiX and sugar syrup (7 days after the last application). The decline in spore counts was maintained throughout the experiment (Table 1).

**Table 1.** Average number of *Nosema* spp. spores in hive bees (HB) x 10<sup>4</sup>

Group	Parameter	Winter hive debris	Average spore count in HB					
			Before treatment	After the administration of treatment and sugar syrup in test No.				
			0/100%	I	II	III	IV	V
N (Nozevit)	$\bar{x}$	13.74	28.0 <sup>A</sup>	9.00 <sup>B</sup>	7.28 <sup>B</sup>	0.72 <sup>B</sup>	0.16 <sup>B</sup>	0.20 <sup>B</sup>
	SD	7.29	22.16	7.30	11.71	0.92	0.17	0.35
AH (Api Herb)	$\bar{x}$	25.52	18.36 <sup>A</sup>	12.64 <sup>a</sup>	3.00 <sup>B</sup>	0.43 <sup>Bb</sup>	0.80 <sup>Bb</sup>	2.65 <sup>B</sup>
	SD	10.15	9.36	14.26	4.85	0.57	1.27	4.36
AX (ApiX)	$\bar{x}$	24.02	20.20 <sup>A</sup>	7.40 <sup>B</sup>	6.84 <sup>B</sup>	1.12 <sup>B</sup>	0.88 <sup>B</sup>	0.32 <sup>B</sup>
	SD	9.47	7.92	7.54	8.38	1.55	1.86	0.41
Cs (syrup)	$\bar{x}$	25.62	23.92 <sup>Aa</sup>	11.48 <sup>b</sup>	11.40 <sup>b</sup>	9.14 <sup>B</sup>	2.60 <sup>B</sup>	1.68 <sup>B</sup>
	SD	14.36	11.62	12.85	4.08	1.22	0.57	0.72
C0	$\bar{x}$	22.90	21.34 <sup>A</sup>	10.72 <sup>Ba</sup>	5.94 <sup>Bcb</sup>	1.32 <sup>Cc</sup>	2.32 <sup>C</sup>	1.06 <sup>Cc</sup>
	SD	9.44	6.98	2.79	2.66	1.40	1.02	0.73

$\bar{x}$  – arithmetic mean, SD – standard deviation. Homogenous groups marked with various letters differ significantly: uppercase letters – at  $p \leq 0.01$ , lowercase letters – at  $p \leq 0.05$

Before treatment, the number of spores in FB was in the range of 10.62 x 10<sup>4</sup> in group AH to 16.14 x 10<sup>4</sup> in group N. During the first test after treatment, the number of spores increased in groups N, AH, AX and Cs, whereas a significant decrease was noted in group C0 (Table 2). Spore counts decreased in all groups on succeeding dates of analysis. The greatest decrease (put the extract number and significance level) was observed in group N, followed by groups AH, C0 and Cs.

A comparison of spore counts in HB and FB in the analyzed groups did not reveal significant differences. A significant increase in the number of spores infecting FB relative to HB was observed only during the first analysis in groups N and AH. A significant increase in the number of spores was observed in HB from group Cs in tests I, II and III, and in HB from group C0 – in test III.

The effectiveness of the analyzed treatments was evaluated by the hemocytometric technique based on the number of spores, but this method was not used to identify *Nosema* pathogens to species level because it does not enable species identification [21]. Microsporidia species were validated by duplex PCR.

**Table 2.** Average number of *Nosema* spp. spores in forager bees (FB) x 10<sup>4</sup>

Group	Parameter	Average number of spores in FB					
		Before treatment	After the administration of treatment and sugar syrup in test No.				
		0/100%	I	II	III	IV	V
N (Nozevit)	$\bar{x}$	16.14	24.60 <sup>a</sup>	6.48 <sup>b</sup>	7.88	0.12 <sup>b</sup>	1.45 <sup>b</sup>
	SD	13.74	24.45	12.17	4.66	0.27	1.88
AH (Api Herb)	$\bar{x}$	10.62	11.36	9.70	2.50	2.30	0.95
	SD	6.57	10.65	14.63	1.45	3.98	1.19
AX (ApiX)	$\bar{x}$	11.70	12.60	2.80	1.20	10.00	5.06
	SD	5.40	3.02	2.85	1.45	21.24	5.90
Cs (syrup)	$\bar{x}$	13.60 <sup>A</sup>	14.96 <sup>A</sup>	4.30 <sup>B</sup>	1.16 <sup>B</sup>	0.20 <sup>B</sup>	1.18 <sup>B</sup>
	SD	6.51	10.51	2.49	1.24	0.20	0.53
C0	$\bar{x}$	13.42 <sup>Aa</sup>	5.53 <sup>b</sup>	5.52 <sup>b</sup>	1.72 <sup>B</sup>	0.28 <sup>B</sup>	2.40 <sup>B</sup>
	SD	3.51	10.36	7.74	1.54	0.18	1.90

$\bar{x}$  – arithmetic mean, SD – standard deviation. Homogenous groups marked with various letters differ significantly: uppercase letters – at  $p \leq 0.01$ , lowercase letters – at  $p \leq 0.05$

Duplex PCR revealed only mixed (*N. ceranae* and *N. apis*) infections (AC) in worker bees from winter hive debris and in HB and FB from all groups before treatment. The species composition identified after treatment during successive tests in both HB and FB from different groups is presented in Tables 3 and 4. A total of 125 HB and FB samples (100%) were analyzed (5 bee colonies per group x 5 sampling dates x 5 groups).

After the treatment HB were most frequently affected by mixed AC infections (59 samples, 47.2%), followed by *N. ceranae* infections (29 samples, 23.2%) and *N. apis* infections (6 samples, 4.8%). Spores were not detected in 31 samples (24.8%). *N. apis* spores were not observed in HB from group Cs fed sugar syrup (Table 3). Forager bees were also most frequently affected by mixed AC infections, which were observed in 56 samples (44.8%). *N. apis* infections were noted in 11 samples (8.8%), and *N. ceranae* infections in 27 samples (21.6%), whereas 31 samples (24.8%) were free of infection (Table 4).

Our research indicates that after treatment the coinfections of *N. apis* and *N. ceranae* were predominant. However, single infections of *N. apis* and *N. ceranae* were also noted. The treatments were not fully effective since spores after treatment were still present, though to a lesser extent. All treatments were more effective in the control of *N. apis* than *N. ceranae*.

Table 3. The effect of treatments on *Nosema* spp. species in hive bees (HB)

Group	Nosema spp. species infecting HB																					
	Before treatment					After treatment in test No.																
	In winter hive debris	I				II				III				IV				V				
AC	AC	AC	A	C	0	AC	A	C	0	AC	A	C	0	AC	A	C	0	AC	A	C	0	
N	5	5	3	-	2	-	3	-	2	-	3	-	1	1	3	-	1	1	2	-	1	2
AH	5	5	3	-	2	-	3	-	2	1	-	1	3	2	1	-	2	1	2	-	2	-
AX	5	5	2	-	2	1	3	1	1	-	3	-	1	1	1	-	1	3	4	-	-	1
Cs	5	5	-	-	4	1	4	-	-	1	2	-	1	2	1	-	3	1	3	-	-	2
C0	5	5	-	-	4	1	3	1	-	1	3	-	1	1	4	-	-	1	2	1	1	1

Table 4. The effect of treatments on *Nosema* spp. species in forager bees (FB)

Group	Nosema spp. species infecting FB																					
	Before treatment					After treatment in test No.																
	In winter hive debris	I				II				III				IV				V				
AC	AC	AC	A	C	0	AC	A	C	0	AC	A	C	0	AC	A	C	0	AC	A	C	0	
N	5	5	4	-	1	-	1	1	3	2	-	2	1	4	-	-	1	2	-	1	2	-
AH	5	5	5	-	-	-	1	-	3	1	1	1	2	1	2	3	-	-	2	1	-	2
AX	5	5	4	1	-	-	2	1	1	1	2	1	1	1	3	-	1	1	-	1	-	4
Cs	5	5	2	1	1	1	2	-	1	2	-	-	3	2	2	-	1	2	3	-	1	1
C0	5	5	4	-	-	1	1	-	3	1	2	-	2	1	3	-	2	-	3	-	-	2

AC – mixed infection (*N. apis*/*N. ceranae*), A – *N. apis* infection, C – *N. ceranae* infection, 0 – no infection.  
 N – Nozevit, AH – Api Herb, AX – ApiX, Cs – syrup, C0 – control

## DISCUSSION

Hemocytometric analyses investigating the effect of Nozevit, Api Herb and ApiX on infected bees revealed that all of the tested treatments lowered *Nosema* spp. spore counts by 31.15% on average. The greatest drop in spore counts was noted in HB treated with Nozevit (67.85%) and ApiX (63.36%). The number of spores in FB increased by 52.41% after the administration of Nozevit and by 7.69% after ApiX treatment. The above results indicate that Nozevit delivers short-lived therapeutic effects. An increase in spore counts was observed in forager bees that need more energy to collect nectar and pollen and are more likely to be exposed to pathogenic spores in the environment.

Similar results were reported by other authors who evaluated the efficacy of different treatments in reducing *Nosema* spp. spore counts. In a study of Sicilian bee colonies displaying evident symptoms of nosemosis, Bessi and Nanetti [22] demonstrated that the administration of Api Herb at 4 g/50 ml of sugar syrup over a period of 3 weeks had reduced the number of *Nosema* spp. spores in worker bees by 46%, whereas fumagillin decreased spore counts by 60%. Valobra *et al.* [23] studied 3 bee groups infected with *Nosema* spp. spores and observed a 71.7% reduction in spore counts in the group administered Api Herb in sugar syrup, 53.6% reduction in the group treated with fumagillin and 30.8% reduction in the control group. According to the cited authors, Api Herb decreases spore counts without exerting an adverse influence on the development of bee colonies. Gajger *et al.* administered Nozevit in sugar syrup and in sugar and honey cake to bee colonies infected with *N. ceranae* spores. Nozevit administered in sugar syrup decreased spore counts by 48.31% after 12 days, by 55.91% after 28 days, by 74.15% after 40 days, and by 81.92% after 60 days. When administered in sugar and honey cake, the resulting drop in spore counts reached 50.63%, 19.25%, 33.42% and 21.10%, respectively. Although *Nosema* spp. infections were not fully suppressed, Nozevit reduced the number of spores in experimental groups relative to the control [24]. In another research Talk- Gajger *et al.* tested the effectiveness of Nozevit. On the 10th day after the treatment of Nosema disease with Nozevit, they observed similar percentage reductions in the number of spores in the treated colonies as in their previous testing. Gajger *et al.* suggested that the herbal preparation induces the production and secretion of mucous from the epithelial layer [17]. Higes *et al.* evaluated the efficacy of Nozevit, using experimental doses (different doses than recommended by the manufacturer). The results showed that in the experimental group after Nozevit treatment of 4 doses a week of 20 drops (1 ml) in 500 ml of sugar syrup, the number of Nosema spores decreased. However, the efficacy Nozevit was lower than when fumagilin was used [18].

In this study, ApiX reduced spore counts in HB by 63.36%, similarly as Nozevit, and it was twice as effective in eliminating nosemosis than Api Herb. A minor increase in spore counts was noted in FB (7.69%), and it was lower than in FB from group Cs fed sugar syrup. The tested treatments did not exert negative effects on bee colonies.

The differences in treatment effect on two *Nosema* species in have never been investigated for various reasons, mainly because the same insect cannot be analyzed on multiple occasions. In this study, the spores were detected microscopically during successive tests in randomly sampled bees. Duplex PCR confirmed the presence of different microsporidia species in worker bees sampled from the same colony on different dates. The above results validate our previous observations [21] that worker bees infected with different *Nosema* species can inhabit the same colony [25]. Meana et al. [26] also demonstrated that the origin of the sampled material plays an important role in the identification of *N. ceranae* spores. In the cited study, spore counts in worker bees differed subject to sampling location and sampling hour. The highest spore counts were reported in forager bees regardless of the sampling hour and in forager and hive bees that were collected around noon.

The treatments used in our research to control *Nosema* infection reduced the load of this pathogen in the treated groups. However, it was not fully effective, because after treatment spores were still present, though less frequently. Based on the presented data we conclude that the Nozevit, Api Herb and ApiX were more efficient to control of *N. apis* compared to *N. ceranae*.

## REFERENCES

1. Paxton R: Does infection by *Nosema ceranae* cause “Colony Collapse Disorder” in Honey bees (*Apis mellifera*)? *J Apic Res* 2010, 49: 80-84.
2. Simeunovic P, Stevanovic J, Vidanovic D, Nisavic J, Radovic D, Stanisic Lj, Stanimirovic Z: A survey of deformed wing virus and acute bee paralysis virus in honey bee colonies from Serbia using real-time RT-PCR. *Acta Vet-Beograd* 2014, 64:81-92.
3. Glavinic U, Stevanovic J, Gajic B, Simeunovic P, Đuric S, Vejnovic B, Stanimirovic Z: *Nosema ceranae* DNA in honey bee haemolymph and honey bee mite *Varroa destructor*. *Acta Vet-Beograd* 2014, 64:349-357.
4. Morse RA, Nowogrodzki R: Honey bee pests, predators and diseases. 1990, Cornell University Press, Ithaca, New York.
5. Gliński Z: Rzedzicki J 1993, *Choroby pszczól*. PWN Warszawa.
6. Webster TC, Pomper KW, Hunt G, Thacker EM, Jones SC: *Nosema apis* infection in worker and queen *Apis mellifera*. *Apidologie* 2004, 35: 49-54.
7. Higes M, Garcia-Palencia P, Martin-Hernández R, Meana A: Experimental infection of *Apis mellifera* honeybees with *Nosema ceranae* (Microsporidia). *J Invertebr Pathol* 2007, 94: 211-217.
8. Forsgren E, Fries I: Comparative virulence of *Nosema ceranae* and *Nosema apis* in individual European honey bees. *Vet Parasitol* 2010, 170:212–217.
9. Milbrath MO, van Tran T, Huang W-F, Solter LF, Tarpay DR, Lawrence F, Huang ZY: Comparative virulence and competition between *Nosema apis* and *Nosema ceranae* in honey bees (*Apis mellifera*). *J Invertebr Pathol* 2015, 125:9–15.
10. Stevanovic J, Simeunovic P, Gajic B, Lalic N, Radovic D, Fries I, Stanimirovic Z: Characteristics of *Nosema ceranae* infection in Serbian honey bee colonies. *Apidologie* 2013, 44:522-536.



11. Czekońska K: The influence of *Nosema apis* on young honeybee queens and transmission of the disease from queens to workers. *Apidologie* 2000, 31: 701-706.
12. Simeunovic P, Stevanovic J, Cirkovic D, Radojicic S, Lakić N, Stanisić L, Stanimirovic Z: *Nosema ceranae* and queen age influence the reproduction and productivity of the honey bee colony. *J Apicult Res* 2014, 53:545-554.
13. Stanimirovic Z, Stevanovic J, Bajic V, Radovic I: Evaluation of genotoxic effects of fumagillin by cytogenetic tests *in vivo*. *Mutation Res-Genet Tox* 2007, 628:1-10.
14. Stevanovic J, Stanimirovic Z, Radakovic M, Stojic V: *In vitro* evaluation of the clastogenicity of fumagillin. *Environ Mol Mutagen* 2008, 49:594-601.
15. van den Heever JP, Thompson TS, Curtis JM, Pernal SF: Stability of dicyclohexylamine and fumagillin in honey. *Food Chem* 2015, 179:152–158.
16. Botias C, Martín-Hernández R, Meana A, Higes M: Screening alternative therapies to control Nosemosis type C in honey bee (*Apis mellifera iberiensis*) colonies. *Res Vet Sci* 2013, 95: 1041-1045.
17. Tlak Gajger I, Kozaric Z, Berta D, Nejedli S, Petrinec Z: Effect of the herbal preparation Nozevit on the mid-gut structure of honeybees (*Apis mellifera*) infected with *Nosema* sp. spores, *Vet Med-Czech* 2011, 56:344–351.
18. Higes M, Gómez-Moracho T, Rodríguez-García C, Botias C, Martín-Hernández R: Preliminary effect of an experimental treatment with “Nozevit®”, (a phyto-pharmacological preparation) for *Nosema ceranae* control. *J Apicult Res* 2014, 53:472-474.
19. OIE (2008) Office International des Epizooties, Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Chap. 2.2.4., Nosemosis of Honey Bees. [http://www.oie.int/fileadmin/Home/eng/Health\\_standards/tahm/2.02.04\\_NOSEMOSIS.pdf](http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.02.04_NOSEMOSIS.pdf)
20. Martín-Hernández R, Meana A, Prieto L, Salvador AM, Garrido-Bailon E, Higes M: Outcome of Colonization of *Apis mellifera* by *Nosema ceranae*. *Appl Environ Microbiol* 2007, 73: 6331-6338.
21. Sokół R, Michalczyk M: Detection of *Nosema* spp. in worker bees of different ages during the flow season. *J Apicult Sci* 2012, 56: 19-25.
22. Bessi E, Nanetti A: Evaluation of three different strategies of *Nosema* control. Abstracts 39<sup>th</sup> Apimondia International Apicultural Congres 21-26 August 2005, Dublin, Ireland.
23. Valobra F, Mandelli U, Bessi E: Abstracts 39<sup>th</sup> Apimondia International Apicultural Congres 21-26 August 2005, Dublin, Ireland.
24. Tlak-Gajger I, Vugrek O, Pinter L, Petrinec Z: „Nozevit pattiens” treatment of honey bees (*Apis mellifera*) for the control of *Nosema ceranae* disease. *Am Bee J* 2009, 149:1053-1056.
25. Michalczyk M, Sokol R, Szczerba-Turek A, Bancercz-Kisiel A. A comparison of the effectiveness of the microscopic method and the multiplex PCR method in identifying and discriminating the species of *Nosema spp.* spores in worker bees (*Apis mellifera*) from winter hive debris. *Pol J Vet Sci* 2011, 14: 385-391.
26. Meana A, Martín-Hernández R, Higes M. The reliability of spore counts to diagnose *Nosema ceranae* infections in honey bees. *J Apicult Res and Bee World* 2010, 49:212-214.

## **EVALUACIJA EFIKASOSTI ODABRANIH TRETMANA INFEKCIJE SA *NOSEMA* SPP. HEMOCITOMETRIJSKOM METODOM I METODOM DUPLEKS PCR**

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U proteklim godinama svedoci smo povećanja mortaliteta pčela u većem broju regiona sveta. Uočeno smanjenje populacije pčela posledica je kombinacije većeg broja faktora pri čemu infekcije sa mikrosporidijama *Nosema apis* i *N. ceranae* daju značajan doprinos ovom fenomenu. Infekcije sa ovim parazitima uslovljavaju skraćenje života pčela i smanjuju produktivnost košnice. Cilj studije je bio evaluacija efikasnosti Nozevit, Api Herb i ApiX (acetilsalicilna kiselina i ekstrakt *Artemisia absinthium* L) u kontroli infekcija prouzrokovanih sa *Nosema* spp, u terenskim uslovima. Posmatrane su dve grupe pčela radilica: radilice u košnici i radilice sakupljačice polena na ulazu u košnicu. Efekti terapija na broj spora kao i vrste mikrosporidija bili su analizirani hemocitometrijskom metodom kao i dupleks PCR metodom. Statistička analiza je pokazala da su tretmani prosečno smanjivali broj spora za 31,15%. Kod radilica u košnici, infekcija sa *Nosema* spp, je najefikasnije smanjena primenom Nozevit (67,85%) i ApiX (63,36%) preparata. Koinfekcija (*N. ceranae* i *N. apis*) je pre tretmana potvrđena u svim uzorcima ispitivanih pčela. Međutim, posle tretmana uočene su infekcija sa pojedinačno *N. apis* i *N. ceranae*. Tretmani koji su testirani, bili su efikasniji u kontroli infekcije sa *N. apis* u poređenju sa kontrolom infekcije *N. ceranae*.