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Laboratory assessment of the effect of nanosilver on longevity, sugar syrup ingestion, and infection of honeybees with Nosema spp.

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Summary

After the withdrawal of Fumagillin, there is no drug which is sufficiently effective against nosema disease. Therefore, intensive research is conducted in order to find new nosemacides. Microsporidia from the genus Nosema are regarded one of the causes of Colony Collapse Disorder (CCD). Hence, any new compound that may be useful in the nosemathosis treatment may be of great importance for veterinary practice.

The aim of the study was to assess the effect of a nanosilver-supplemented diet on worker-bee longevity and on the level of worker-bee infection with Nosema spp. in honeybees in cage tests.

The diet supplemented with 25 ppm of nanosilver decreased the number of nosema spores. Therefore the compound might be considered as useful in the nosemosis disease therapy.

On the other hand, in experiment I, supplementation of the syrup with 25 ppm of nanosilver significantly shortened worker-bees' lifespan. This, however, was not observed in experiment II. Honeybees fed with syrup supplemented with 25 ppm of nanosilver consumed the greatest amounts of the syrup. Moreover, bees fed with syrup supplemented with 12.5 and 25 ppm of nanosilver exhibited increasing contents of silver in their bodies.

Keywords: Nosema spp., nanosilver, laboratory test, sugar syrup, bee longevity, XRF

After the withdrawal of Fumagillin, there is no drug which is sufficiently effective against nosema disease. Therefore, intensive research is conducted in order to find new nosemacides. Microsporidia from the genus *Nosema* are regarded as one of the causes of Colony Collapse Disorder – CCD (6). Hence, any new compound that may be useful in nosemathosis treatment may be of great importance for veterinary practice.

Nosema apis causes disease symptoms that are visible particularly in spring. Beekeepers have learned how to control this type of nosemathosis. However, honeybees are being infested by a new parasite, *Nosema ceranae*, which was first detected as a pathogen of the eastern honeybee *Apis cerana* inhabiting warm-climate zones (3). The pathogen has adapted to a cooler climate and a new host, i.e. *A. mellifera*. Disease symptoms caused by *N. ceranae* are visible in the second half of summer. Moreover, the pathogen exhibits higher virulence than *N. apis*, and honeybees cannot overcome infections from this endoparasite (4, 5, 16). Nowadays, two *Nosema* species that infect honeybees can be identified by molecular analyses (2, 11) or under a scanning microscope. Mixed infections with predominance of *N. cerance* are the most prevalent in Poland (15).

Hence, new formulations are being developed to control Colony Collapse Disorder and to improve bee health. One of these is nanosilver and its solutions, which exhibit bactericidal, virucidal, and fungicidal activity. Silver blocks the respiratory chain and, in fungi, inhibits water binding during the developmental cycle (12). Silver molecules in nanosilver have a metallic form with a small particle size in a range from 1.5 to 5 nm. This facilitates the formation of conglomerates, which allows nanoparticles to encapsulate and penetrate into the pathogen, thereby limiting their development in the host organism (7-9, 12).

The aim of the study was to assess the effect of a nanosilver-supplemented diet on worker-bee longevity and on the level of worker-bee infection with *Nosema* spp. in honeybees in cage tests.

Material and methods

Two experiments were conducted on worker-bees originating from one mother-queen. A comb with a brood in the 20th day of a development was put into a chamber room and kept at a constant temperature and humidity (36°C, 65%). Bees emerged during the 21st day. One-day-old workers were placed in wooden cages. The cages had glass front screens, as well as ventilating and feeding slots (1).

In Experiment I, two groups were maintained:

- control – the bees were fed with water : sugar syrup at a 1:1 proportion;
- nanosilver 25 ppm – the bees were fed with water : sugar syrup (1:1) with the addition of 25 ppm of nanosilver.

In Experiment II, four groups were maintained:

- control – the bees were fed with water : sugar syrup (1:1);
- control + *Nosema* spp. – from the 1st to the 4th day of the experiment, the bees were fed with sugar : water syrup (1:1) prepared with water containing approximately 8×10^6 *Nosema* spp. spores. After the 4th day, the bees were supplied with pure water : sugar syrup (1:1);
- nanosilver 12.5 ppm + *Nosema* spp. – from the 1st to the 4th day of the experiment the bees were fed with sugar : water syrup (1:1) prepared with water containing approximately 8×10^6 *Nosema* spp. spores. After the 4th day, the bees were supplied with water : sugar syrup (1:1) with 12.5 ppm of nanosilver/1 ml of the syrup;
- nanosilver 25 ppm + *Nosema* spp. – from the 1st to the 4th day of the experiment, the bees were fed with sugar : water syrup (1:1) prepared with water containing approximately 8×10^6 *Nosema* spp. spores. After the 4th day, the bees were supplied with pure sugar syrup at the 1:1 proportion with 25 ppm of nanosilver/1 ml syrup.

In both experiments, each group consisted of 12 cages, 50 workers in each. The cages were kept in an air-conditioned chamber at a temperature of 26°C and ca. 60% relative humidity. Dead workers were removed daily from each of the cages. Microscopic preparations were made from each individual dead worker-bee and the *Nosema* ssp. spores were counted in a Bürker chamber in five vision fields (10). In experiment II, silver residues in bees that died respectively at the age of 1-day, 20-day and 30-day were detected with the X-ray fluorescence spectrophotometry (XRF). Six replicates of the test were performed for each age-group within each nanosilver concentration (control, control + *Nosema* spp., nanosilver 12.5 ppm + *Nosema* spp., nanosilver 25 ppm + *Nosema* spp.).

The results were statistically analysed with the SAS software (SAS Institute 2002-2003 SAS/STAT User's Guide Version 9.13, Cary, NC, Statistical Analysis System Institute) using the one-way ANOVA (a group effect was the experimental factor) and Tukey's HSD (honestly significant difference) test (14).

Results and discussion

The diet supplemented with 25 ppm of nanosilver decreased the number of *Nosema* spp. spores (Tab. 1). Therefore the compound might be considered as useful in the nosemosis therapy.

On the other hand, in experiment I, supplementation of the syrup with 25 ppm of nanosilver significantly shortened worker-bees' lifespan (Tab. 2). This, however, was not observed in experiment II (Tab. 2). Honeybees fed with syrup supplemented with 25 ppm of nanosilver consumed the greatest amounts of the syrup (Tab. 2). Moreover, bees fed with syrup supplemented with 12.5 and 25 ppm of nanosilver exhibited increasing contents of silver in their bodies (Tab. 3). The prolonged period of consumption of syrup with nanosilver was accompanied by an increased (even 167-fold) content of silver in bee bodies (6.66/0.04 = 167) (Tab. 3).

Roman (12), Roman and Chorbiński (13) has demonstrated a low cidal activity of nanosilver against *Ascosphaera apis*. This does not correspond to our results in *Nosema* spp. Similar to our research in *Nosema* spp., Roman (12) Roman and Chorbiński (13)

Tab. 1. Number of *Nosema* spp. spores in infected bees (mln)

Group	Mean	SE	Min.	Max.
Experiment I				
Control	11.36[b]	1.12	0	336
Nanosilver 25 ppm	3.72[a]	0.92	0	480
Experiment II				
Control	10.52[b]	1.44	0	320
Control + *Nosema* spp.	10.36[b]	1.04	0	716
Nanosilver 12.5 ppm + *Nosema* spp.	10.24[b]	0.84	0	560
Nanosilver 25 ppm + *Nosema* spp.	6.64[a]	1.04	0	440

Explanation: a, b, c – different letters in columns indicate statistically significant differences between the groups ($p < 0.05$); SE – standard error; Min. – minimum trait values; Max. – maximum trait values

Tab. 2. Life-span and food intake in worker-bees that consumed nanosilver

Group	Longevity expressed as numbers of days counted from the beginning of the test to the day on which 75% of worker bees survived, respectively	50%	25%	0%	Average dailyfood intake (µl)
Experiment I					
Control	13	19[b]	28[b]	45[b]	67.6[a]
Nanosilver 25 ppm	11	15[a]	20[a]	34[a]	82.9[b]
Experiment II					
Control	8	11	17[ab]	28	86.3[a]
Nontrol + *Nosema* spp.	8	10	14[a]	27	80.8[a]
Nanosilver 12.5 ppm + *Nosema* spp.	8	11	14[a]	29	81.8[a]
Nanosilver 25 ppm + *Nosema* spp.	9	12	18[b]	28	104.5[b]

Explanation: a, b, c – different letters in columns indicate statistically significant differences between the groups within the experiment ($p < 0.05$)

Tab. 3. Silver residue (mg/100 mg dw) in one worker-bee determined by XRF, experiment II

Group	Content of silver in bees which died at the age		
	1 day	20 days	30 days
Control	0,03	0,03	0,03
Control + *Nosema* spp.	0,04	0,04	0,04
Nanosilver 12.5 ppm + *Nosema* spp.	0,04[a]	2,13[b]	3,38[c]
Nanosilver 25 ppm + *Nosema* spp.	0,04[a]	4,25[b]	6,66[c]

Explanation: a, b, c – different letters in rows indicate statistically significant differences between the groups ($p < 0.05$)

also showed an increased concentration of the silver molecules in bee bodies (even up to a 71-fold increase) and in honey (even 24-fold).

Therefore, a possible application of nanosilver in prophylaxis and treatment of nosemosis may result in the contamination of bee products. Accumulation of silver in bee bodies may also lead to contamination of bee products *via* enzymes produced by worker-bees.

Conclusions

Although supplementation of the honeybee diet with nanosilver reduces *Nosema* spp. infection, it causes deposition of silver in bee organisms. The accumulation of silver in the bee organisms suggests that nanosilver in veterinary and medical practice should be used with caution.

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