COMPARISON OF FUMAGILLIN AND Nonosz® AGAINST *N. ceranae* NOSEMOSIS OF THE HONEYBEE (*Apis mellifera* L.)

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SUMMARY

Nosemosis caused by *Nosema apis* has been regarded as an important honeybee disease for a long time. At present, issues related to its prevention are again in the focus of apiarists' interest all over the world. The anxiety caused by honeybee losses observed in many places, coupled with the presence of a new species (*Nosema ceranae*) recently introduced into and detected in many European countries, adds to the uncertainty of control.

In this study we compared the efficacy of fumagillin, a compound of antibiotic type and known to be effective against *N. apis*, is compared with that of Nonosz®, a product of different type. Our results document that fumagillin can be used successfully against *N. ceranae*, and the use of Nonosz® may have an alternative in the control of this parasitose.

ÖSSZEFOGLALÁS

Szalainé Mátray E. – Harka L. – Hegedűs D. – Albert A. – Békési L.: A FUMAGILLIN ÉS A Nonosz® ÖSSZEHASONLÍTÁSA A MÉZELŐ MÉH (*Apis mellifera* L.) *Nosema ceranae* OKOZTA NOSEMOSISÁBAN

A *Nosema apis* okozta nosemosis a mézelőméh egyik fontos betegsége. Újabban a betegség elleni védekezés ismét a méhészek által gyakran felvetett kérdések közé került az egész világon. A sokfelé tapasztalt méhpusztulások, együtt az új kórokozó faj (*Nosema ceranae*) megjelenésével, csak fokozza a védekezés bizonytalanságát.

Ebben a vizsgálatban a *N. apis* ellen ismerten hatékony szer, a fumagillin antibiotikum hatását hasonlítottuk össze egy más jellegű szerrel a Nonosz® -szal. Eredményeink bizonyítják, hogy namcsak a fumagillin lehet hatékony a *N. ceranae* ellen, hanem alternativ szerként a Nonosz® -t is lehet használni a nosemosis kezelésére.

INTRODUCTION

Nosema apis Zander, the causative agent of nosemosis of the honeybee (*Apis mellifera* L.), is a parasitic fungus belonging to the class Microsporea (Cnidosporidia, Sporozoa). This protozoan parasitizes the midgut epithelial cells of adult bees. Its spores measuring 5×7 μm and having characteristic structure are shed by infected bees with their excrement of altered consistency (Koltai, 1985). Another microsporidian species, *Nosema ceranae* Fries, was reported from the Asian honeybee (*Apis ceranae* Fabricius), and has been shown to be able to infect the European honeybee as well. Later on *N. ceranae* infection was diagnosed in Asia, and then in 2006 the presence of *N. ceranae* in *A. mellifera* was reported for the first time in Europe, from Spain (*Higes és mtsai*, 2006). Subsequently it was detected in several European countries, and in 2007 its presence in Hungary was documented by Hungarian authors who stated that nosemosis of honeybees is caused primarily by this parasite species also in Hungary (*Tapaszti és mtsai,* 2009).

Paxton és mtsai (2007) suggest that *N. ceranae*, which is probably more virulent than *N. apis*, has been present in Europe at least since 1998. This species is thought to have higher

pathogenicity as a certain part of the spores released from the necrotic epithelial cells more commonly cause autoinfection. As on the basis of the usual spore morphology it is impossible to distinguish the two species, a rapid PCR-RFLP procedure has been developed for this purpose in Hungary as well (*Tapaszti és mtsai*, 2009).

Because of the high honeybee density, practically all apiaries can be considered infected in Europe, i.e. *Nosema* spores are ubiquitous, although the disease develops only if predisposing factors are present. It is an alarming fact that nowadays our bee colonies are debilitated by a broad range of factors including pollen deficiency, mild toxicoses, *Varroa* mite infestation and the acaricidal treatment used to control it, virus infections, and technological stress (*Békési*, 2008). Therefore, nosemosis may be an important component of the Colony Collapse Disorder decimating honeybees all over the world.

Katznelson és Jamieson (1952) had established more than 50 years ago that the antibiotic fumagillin, derived from the fungus *Aspergillus fumigatus*, effectively inhibited the development of *Nosema* parasites. *Williams és mtsai* stated in 2008, that fumagillin temporarily does control *N. ceranae* either. Because of its antibiotic character the registration of this compound for bees has been generally withdrawn in the countries of the European Union, and for several years it has not been available on the Hungarian market either. In view of the risk of disease outbreaks due to the appearance of the new *Nosema* species, in the autumn of 2008 Fumidil B AUV (CEVA-Phylaxia, Budapest, Hungary) containing fumagillin as active ingredient was put on the Hungarian market with a case-by-case marketing authorisation. As the use of antibiotics in food-producing animals is subject to increasingly stringent regulation, the possibility of their replacement with other products of non-antibiotic nature is a much discussed subject.

Nonosz® (recently modified as Nonosz Plus), a product having curative effect but not qualifying as a drug (containing the sodium salt of ortho-hydroxy-carbonic acid and *Beta vulgaris* cv.), was developed in Hungary and, based upon the positive results of preliminary trials executed by beekeepers, was put into circulation for nosema prevention (TolnAgro Ltd., Szekszárd, Hungary). However, studies comparing its efficacy with that of fumagillin and test results on its effect on the new species *N. ceranae* were lacking.

MATERIALS AND METHODS

In the experiments reported here, the two products were tested under laboratory conditions and in five apiaries in the spring of 2009.

In the laboratory experiment, 10 young adult bees per group, derived from bee colonies neither showing signs of *Nosema* infection nor spore shading, were infected individually with 10^5 *N. ceranae* spores. Before infection, the bees were fasted for 2 hours and then were fed on thick honey after treatment. After the infection had taken (5 days), one group each was treated with a therapeutic dose of Fumidil B and Nonosz®, respectively, administered in sugar syrup every other day, on a total of five occasions. Non-infected and infected, non-treated groups were used as control. The cages were kept in thermostat at 25 °C temperature and 90% relative humidity. Dead bees were removed and examined daily. On the second day after the last treatment (on day 12 of the experiment) the experiment was terminated and the remaining bees were examined individually for *Nosema* parasites.

For the field trials, we first examined 30 bee colonies each in five apiaries by taking samples from the outgoing bees. On the basis of the *Nosema* examination, we formed three groups of the same infection status, with five bee colonies per group, 15 of the 30 colonies. Examination for *Nosema* infection was done by homogenizing 30 bees each in 30 ml of distilled water in a braying mortar. From the homogenate, one drop each was placed on a slide, covered with a coverslip, and the samples were examined under microscope using an objective of 40-fold magnification. The severity was scored on the basis of relative spore quantity (easy to judge by examiners having some experience) from one to three crosses (+). Occasionally, to check the results, we performed spore counting by the method of *Cantwell* (1970), using a *Fuchs-Rosenthal* cell counting chamber. Three of the selected apiaries had to be excluded because of the low infection rate, and thus only two apiaries (Apiary 1 and Apiary 2) proved to be suitable for the field trials.

The treatments were performed by the method of sugar syrup trickling. The content of one 25-g bottle of Fumidil B was divided into five equal parts, one dose was dissolved in 250 ml of sugar syrup in 1:1 ratio, and then, using a syringe, 50 ml volumes of that solution were trickled onto the top of the frame bars or on the honeybees in the rows of the honeycomb cells. The treatment was repeated every other day, on a total of five occasions, preparing a fresh solution for each treatment. Two hundred millilitres of Nonosz® were mixed into 1800 ml of sugar syrup. The solution was stored in a cool place, and 50 ml volumes each were trickled onto the selected colonies every other day, on a total of five occasions. The control colonies were treated by trickling onto them 50 ml sugar syrup each every other day. On the day after the last treatment, honeybee samples were taken from both the treated colonies and the five controls for *Nosema* examination.

The relative spore counts obtained before and after the treatment for the honeybees participating in the laboratory experiment and for the individual honeybee colonies (the number of positive bees and colonies) were added up, and the results of treatment were evaluated on the basis of the total scores.

RESULTS

The results of trials conducted with the bee colonies in the spring of 2009 are presented in *Tables 1 and 2*.

Table 1

Results of the treatment trial conducted on 15 test colonies of Apiary 1

Fumidil B		**Nonosz**		**Control**	
Initial stage (1)	**After treatment (2)**	**Initial stage**	**After treatment**	**Initial stage**	**Closing stage (3)**
155 +++(4)	Neg.	172 - +++	+	59 - +++	++
156 - +++	+	176 - +++	Neg.	65 - +++	+
162 - ++	Neg.	180 - +++	++	51 - ++	++
167 - ++	+	174 - +	Neg.	55 - ++	+++
168 - +	Neg.	179 - +	Neg.	58 - +	Neg.
In toto 11+	**2+**	**11+**	**3+**	**11+**	**8+**

Crosses (+) indicate the severity of infection, while total infection denotes the cumulative severity of infection in the different groups on the basis of the relative spore counts (4). At the head of the columns the numbering of the bee colonies is indicated

1. Táblázat Az 15 méhcsaládon végzett kezelési kísérlet eredménye (1. sz. Méhészet).

A keresztek száma (+) a fertőzöttség intenzitását mutatja és a keresztek összege a fertőzöttség kumulativ értékét jelzi a spóraszámok alapján az egyes csoportokban (4). Az első oszlopban a méhcsaládok jelzése van feltüntetve.

kezdő állapot (1), kezelés után (2), záró állapot (3)

Table 2

Comparison of the development of spore infection in the 15 test colonies (Apiary 2).

Fumidil B		**Nonosz**		**Control**	
Initial stage (1)	**After treatment (2)**	**Initial stage**	**After treatment**	**Initial stage**	**Closing stage (3)**
2+++ (4)	++	3 - +++	++	13 - +++	+++
18 - +++	+	24 - +++	+	8 - ++	++
11 - ++	Neg.	16 - ++	Neg.	25 - ++	++
1 - +	++	5 - +	+	14 - +	Neg.
15 - +	Neg.	6 - Neg.	+	12 - Neg.	++
Összes 10+	**5+**	**9+**	**5+**	**8+**	**9+**

Crosses (+) indicate the relative severity of infection, while total infection denotes the cumulative severity of infection in the different groups (4). At the head of the columns the numbering of the bee colonies is indicated

2. Táblázat A 15 méhcsaládon végzett kezelési kísérlet eredményének összehasolítása (2. sz. Méhészet).

A keresztek száma (+) a fertőzöttség intenzitását mutatja és a keresztek összege a fertőzöttség kumulatív értékét jelzi a spóraszámok alapján az egyes csoportokban (4). Az első oszlopban a méhcsaládok jelzése van feltüntetve.

kezdő állapot (1), kezelés után (2), záró állapot (3)

No abnormality or mortality was observed in the treated bee colonies in the field trial. The results of the laboratory experiment are shown in Figure 1.

Figure 1

Results of the laboratory experiment conducted with artificially infected, caged honeybees, with the indication of spore counts.

1. *ábra A zárkázott, mesterségesen fertőzött méhekkel végzett laboratóriumi kísérlet eredménye a relativ spóraszámok alapján*

Among the honeybees treated with Nonosz®, mortality occurred in the first days when the infection rate of the bees was still high.

DISCUSSION

Nosema-infected honeybees hardly show any clinical signs: at most their abdomen might appear swollen. Traces of excrement may be seen throughout the hive as well as on the flight board; this is usually not seen in the case of *N. ceranae* infection. The lifespan of heavily infected bees may be reduced to half, which may result in slow development of the colony in the spring and its depopulation in the summer (*Koltay*, 1985). The functioning of the pharyngeal gland may also be impaired, as a result of which 15% of the eggs will fail to develop into viable larvae (*Bailey és Ball*, 1991). Infection decreases the amount of vitellogenin (winter reserve complex) in the fat bodies, thus lowering the overwintering chances of infected bees. Nosemosis is the most insidious pathogen in apiculture all over the world.

Williams és mtsai (2008) suggested that fumagillin was successful at temporarily reducing spore numbers of *N. ceranae*, this recent invasive parasite in European honey bees. In our present experiment, the two products used for the therapy of nosemosis showed practically the same efficacy: both agents markedly reduced spore production as compared to the untreated controls. The results are presented in *Tables 1* and *2*. Comparing the results of the treatment trial conducted in the two apiaries based upon the average values of 10 bee colonies, it can be stated that Nonosz® used by trickle treatment five times reduced the spore count at least as effectively or even more than did Fumidil B: during the ten-day period the spore count decreased to about one-third of the initial spore count, while in the untreated controls it hardly changed at all (*Figure 2*) or due to the mild climate spontaneous decrease of spore production was noticed.

Figure 2

The sum of relative spore counts found in bee colonies of the two apiaries before and after treatment (number of + results) as compared to infection of the untreated controls

2. ábra A relative spóraszámok összege a két méhészet vizsgált méhcsaládjaiban a kezelés előtt és után (a fertőzöttség erősségét jelentő +-ek összege), összehasonlítva a kezeletlen kontrollokkal.

Evaluation of the results of the laboratory experiments indicates that artificial *N. ceranae* infection of individual honeybees initiated spore production in all cases. As a result of Fumidil B treatment, there were hardly any honeybee losses and spore production remained on a low level. In the honeybees treated with Nonosz®, the beneficial effect of treatment occurred somewhat later. In the first days after treatment the bee losses in the Nonosz®-treated group were on a similar level as in the controls, but the overall spore production was lower (*Figure 1*).

Based upon the results of the laboratory experiments and field trials, it can be stated that Nonosz® was similarly effective as fumagillin in arresting spore production in the case of artificial and natural *N. ceranae* infection (tests conducted at the Department of Microbiology and Infectious Diseases, Faculty of Veterinary Science, Szent István University demonstrated the presence of *N. ceranae* in the bee colonies of both apiaries).

It is known that fumagillin arrests only the intracellular development cycle of the parasite and does not affect the viability of spores. The mechanism of action of Nonosz® and its effect exerted on the spores should be the subject of further studies.

The present study did not involve the measurement of possible drug residues, if any, left behind either in the bees or in the hive (honey or wax).

The treatment must be complemented with the elimination of predisposing factors as well as disinfection against spores with acetic acid in all cases.

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