

## Testing *in Vitro* of an Apifitoterapeutic Formula Against *Nosema spp.*

Vasilica Savu<sup>1</sup>, Ion Radoi<sup>2</sup>, Maria Măgdici<sup>1</sup>, Silvia Pătruică<sup>3</sup>, Nicoleta Ion<sup>1</sup>, Agripina Sapcaliu<sup>1</sup>

<sup>1</sup>Beekeeping Research and Development Institute of Bucharest, 011464, Bucharest, 42 Ficusului Blvd, Romania

<sup>2</sup>University of Agronomical Sciences and Veterinary Medicine Bucharest

<sup>3</sup>Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Calea Aradului, 119, 300645, Timisoara, Romania

---

### Abstract

Nosema, a parasitic disease that affects adult honey bees, has a directly correlation with the losses of bee colonies, until to depopulation. The target of our study was to determine the antinosema action of an apifitoterapeutic formula that was obtained in an earlier phase of our researches. In the present study, we have had two experiences (F and N) formed by clinically healthy bees. The experimental bees have received, *in vitro*, naturally infested honey (7 spores by *Nosema spp* / field). The first experience (F, I-IX groups) was treated with apifitoterapeutic formula (10 ml/ honey kg), for 10 days (from T<sub>1</sub> to T<sub>2</sub> moment), while the second experience (N, with X-XVIII groups) was infested with naturally infested honey, for 20 days (from T<sub>1</sub> to T<sub>2</sub> moment). The first experience (F) showed 22% positive diagnosed bees, while the second experience (N) showed 89% positive diagnosed bees. In the first experience, the infestation degree was very weak (group I) and weak (group III), while the other groups were negative. The antiparasitic formula has showed, in laboratory conditions, a positive impact on experimental honey bees, with an efficiency over 78%. In the further, testing prophylactically and therapeutically will be conducted on bee families.

**Keywords:** apiphytotherapy, honey bee, *Nosema spp.*, spores

---

### 1. Introduction

Nosemosis (nosema) is a honey bee disease caused by two species of protozoan of *Nosema spp* (*N. apis* and *N. ceranae*), present both in Romania and in other neighbour European countries resulting in the depopulation of bee colonies [1-7]. The purpose of the paper is to determine *in vitro* the action of *anti-Nosema spp.*, an original apiphytotherapeutic formula, obtained in a previous stage of research, based on hydro-alcoholic extracts of plants and propolis.

### 2. Materials and methods

The experiments were performed on 2 lots of clinically healthy bees, initially evaluated by O.I.E. methodology used to diagnose nosema, which received naturally infested honey at an average of 7 spores of *N. apis/N. ceranae*/camp. The honey infestation degree was determined by a complex algorithm formulated according to the O.I.E methodology, and spore counting was achieved by means of a 40 microscopic lens [8]. The age of bees in the experiment was an average of 10 days when the experiment started (T<sub>0</sub>), and they were kept at a 30 + 3 C temperature in identical conditions for both lots, in Fotti box. After 10 days since the beginning of the experiment (T<sub>1</sub>) both lots were evaluated by quantifying the spore number, infestation

---

\* Corresponding author: Agripina Sapcaliu,  
[sapcaliuagripina@yahoo.com](mailto:sapcaliuagripina@yahoo.com)

degree, mortality percentage and number of alive and dead bees. Spores were counted by use of a 400 magnitude Burker-Turk haemocytometer, and the differentiation of spore types was made based on their morphological characteristics, which caused the suspicion that the two *Nosema spp.* species existed [5]. At T<sub>1</sub> moment, infestation by *Nosema spp.* was certified and the infestation degree was evaluated in the analyzed samples. The experiment continued for 10 days until T<sub>2</sub> moment (20 days since the experiment started) by administering the apiphytotherapeutic formula (10

ml/kg of honey) in groups I – IX (experimental lot F) while groups X-XVIII (experimental lot N) received only infested honey for 20 days since T<sub>0</sub> moment.

### 3. Results and discussion

Upon examination at T<sub>1</sub> moment, after 10 days, the infestation evolution and the infestation degree in analyzed samples are presented in Table 1.

**Table 1.** Diagnosis and infestation degree in analyzed samples of *Nosema spp.* in the experimental lot at T<sub>1</sub> moment (10 days)

GROUP	COLONY STATE (T <sub>0</sub> moment)	Infestation degree after 10 days (T <sub>1</sub> moment)				Nosema suspicion type N. a / N. c.	Mortality T <sub>1</sub> moment (%)	Dg	Bees (40 pieces) alive/ dead
		Very low (1-2)	Low (2-10)	Average (11-20)	Massive ≥ 21				
1	Clinically healthy	-	+	-	-	N. c.	*	Positive	18/22
2	Clinically healthy	+	-	-	-	N. a	**	Positive	29/11
3	Clinically healthy	-	-	-	+	N. a	**	Positive	25/15
4	Clinically healthy	-	-	-	-	-	*	Negative	14/26
5	Clinically healthy	-	-	-	-	-	**	Negative	22/18
6	Clinically healthy	+	-	-	-	N. c.	**	Positive	19/21
7	Clinically healthy	+	-	-	-	N. a/N. c.	**	Positive	30/10
8	Clinically healthy	+	-	-	-	N. a/N. c.	**	Positive	33/7
9	Clinically healthy	-	-	-	-	-	*	Negative	6/34
10	Clinically healthy	+	-	-	-	N. c.	*	Positive	19/21
11	Clinically healthy	-	+	-	-	N. a/N. c.	*	Positive	16/24
12	Clinically healthy	-	-	-	+	N. a/N. c.	**	Positive	24/16
13	Clinically healthy	-	-	-	-	-	*	Negative	5/35
14	Clinically healthy	-	-	+	-	N. a/N. c.	**	Positive	27/13
15	Clinically healthy	+	-	-	-	N. a/N. c.	**	Positive	22/18
16	Clinically healthy	-	-	-	+	N. c.	*	Positive	17/23
17	Clinically healthy	-	-	-	-	-	*	Negative	9/31
18	Clinically healthy	-	-	-	-	-	*	Negative	5/35
<b>Total positive groups (%)</b>		<b>6 (33%)</b>	<b>2 (11%)</b>	<b>1 (6%)</b>	<b>3 (17%)</b>		<b>12 (67%)</b>		
<b>Total negative groups</b>							<b>6 (33%)</b>		

N. a = *Nosema apis*; N. c = *Nosema ceranae*; \* ≤ 50 % mortality ; \*\* 50-80 % mortality; \*\*\*81-100 % mortality

According to Table 2, 20 days after the experiment started, the experimental lot F (groups I-IX) presented a percentage of 22% bees diagnosed positive (group I and III) having a very

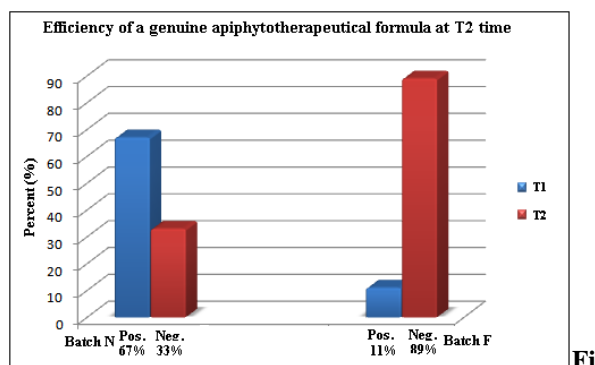
low and low infestation degree and 78% diagnosed negative. The experimental lot N (*nosema*) presented upon T<sub>2</sub> moment a percentage of 89% bees diagnosed positive.

**Table 2.** The therapeutic efficiency of the original apiphytotherapeutic formula at T<sub>2</sub> moment

GROUP	COLONY STATE (T <sub>0</sub> moment)	Infestation degree upon T <sub>2</sub> moment (20 days)				Nosema suspicion type N. a / N. c.	Mortality T <sub>1</sub> moment (%)	Dg	Bees (40 pieces) alive/ dead
		Very low (1-2)	Low (2-10)	Average (11-20)	Massive ≥ 21				
		1	Clinically healthy	+	-				
2	Clinically healthy	-	-	-	-	-	**	Negative	30/11
3	Clinically healthy	-	+	-	-	N. a	**	Positive	30/10
4	Clinically healthy	-	-	-	-	-	*	Negative	18/22
5	Clinically healthy	-	-	-	-	-	**	Negative	24/26
6	Clinically healthy	-	-	-	-	-	**	Negative	21/19
7	Clinically healthy	-	-	-	-	-	**	Negative	32/8
8	Clinically healthy	-	-	-	-	-	**	Negative	35/5
9	Clinically healthy	-	-	-	-	-	*	Negative	6/34
10	Clinically healthy	-	-	-	-	N. c.	***	Positive	4/36
11	Clinically healthy	-	-	-	-	N. a/N. c	***	Positive	2/38
12	Clinically healthy	-	-	-	-	N. a/N. c	***	Positive	9/31
13	Clinically healthy	-	-	-	-	-	*	Negative	30/10
14	Clinically healthy	-	-	-	-	N. a/N. c	**	Positive	11/29
15	Clinically healthy	+	-	-	-	N. a/N. c	**	Positive	15/25
16	Clinically healthy	-	-	-	-	N. c	***	Positive	6/34
17	Clinically healthy	+	-	-	-	-	*	Positive	27/13
18	Clinically healthy	-	-	-	-	-	**	Negative	11/29

N. a = Nosema apis; N. c = Nosema ceranae, \* ≤ 50 % mortality, \*\* 50-80 % mortality, \*\*\*81-100 % mortality

Figure 1 presents graphically the therapeutic efficiency of the original apiphytotherapeutic formula at T<sub>2</sub> moment.



**Figure 1.** Comparative aspects of the therapeutic efficiency of the original apiphytotherapeutic formula at T<sub>2</sub> moment

#### 4. Conclusions

The experimental infection in bees caused by the administration for 20 days of naturally infested honey was certified at 89% of the untreated experimental lot.

The original apiphytotherapeutic formula based on hydro-alcoholic extracts of plants and propolis was efficient in the treatment of the *Nosema spp.* infection in 78% of the lot that was treated by 10 ml formula/ kg of honey. Laboratory results recommend using the apiphytotherapeutic formula

as a therapeutic alternative to traditional veterinary medicine after subsequent clinical testing.

#### Acknowledgements

The research was supported by project *PN 108/2012* "Studies on the preparation and testing of an apiphytotherapeutical product for veterinary use "NOSEMA-API" for the treatment and prophylaxis of *Nosema* disease in bee families"

#### References

- 1 Botías C., Martín-Hernández R., Garrido-Bailón E., GonzálezPorto A., Martínez-Salvador A., Pilar De La Rúa, Meana A., Higes M. The growing prevalence of *Nosema ceranae* in honey bees in Spain, an emerging problem for the last decade. Res. Vet. Sci., 2011, DOI: 10.1016/j.rvc.2011.08.002
- 2 Chauzat M.P., Higes M., Martin-Hernandez R., Meana A., Cougoule N., Faucon J.P. Presence of *Nosema ceranae* in French honey bee colonies. J. Apicult. Res. 2007, 46, 127-128
- 3 Fries I. *Nosema ceranae* in European honey bees (*Apis mellifera*). J. Intervertebr. Pathol. 2010, 103, 573-579.
- 4 Forsgren E., Fries I. Comparative virulence of *Nosema ceranae* and *Nosema apis* in individual European honeybees. Veterinary Parasitology, 2010, 170, 212-217.
- 5 Giersch T., Berg T., Golea F., Hornitzky M. *Nosema ceranae* infects honeybees (*Apis mellifera*) and

contaminates honey in Australia. *Apidologie*, 2009, 40, 117-123.

6. Paxton R., Kee J., Korpela S., Fries I. *Nosema ceranae* has infected *Apis mellifera* in Europe since at least 1998 and may be more virulent than *Nosema apis*. *Apidologie* 2007, 38, 558-565.

7. Tapaszti, Z., Forgách P., Kövágó C., Békési L., Bakonyi T., Rusvai M. First detection and dominance

of *Nosema ceranae* in Hungarian honeybee colonies. *Acta Veterinaria Hungarica*, 2009, 57, 383-388.

8. OIE. Manual of diagnostic tests and vaccines for terrestrial animals, Chapter 2.2.4. Nosemosis of Honey Bees. Office International des Epizooties; Paris, France, 2008.