THE IMPACT OF CONTAMINATION WITH *NOSEMA SSP.* SPORES ON HONEY OBTAINED BY APIS MELLIFERA CARPATHICA

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Abstract

Nosemose disease is a parasitic disease that affects old honey bees. It is produced by ubiquitous and opportunistic germs of Nosema spp., coupled with huge losses of honey bees within colonies (by depopulation), and reducing of honey production. In Romania, nosemose disease was officially admitted as being produced by two species of Nosema (Nosema apis and Nosema ceranae). The aim of our study was to establish a possible correlation between the honey naturally infested with spores of Nosema spp. (from families diagnosed positive), and the quality of honey used for human consumption. The study was performed on 65 canola honey samples received from private apiaries, of which 40% of them were taken and analyzed (26 positive samples). Various microscopic analyzes, organoleptic and physicochemical on the properties of honey samples were made. The results showed us that there were significant changes in the honey quality correlated with the degree of its natural pollution. We were found that the honey samples with more than 5 spores of Nosema spp./experimental field have presented serious deterioration in terms of organoleptic and physical-chemical properties. We grouped honey samples (26 samples) into 3 categories, according with their physicochemical and organoleptic changes. These changes in the honey quality have a negative economic impact on the use of bee products, and on health of bee families, too.

Keywords*: Apis mellifera, honey bee quality, Nosema spp. spores*

Introduction

Nosemoses are parasitoses frequently caused by two species of microsporidia belonging to the *Nosema spp.* (*N. apis* and *N. ceranae*) usually considered to be ubiquitary and opportunistic germs. Infections become endemic in countries that have long and humid winters, when climate factors favor them, as in the case of our country (5,6). The negative effects are to be found in various forms in bee colonies: diarrhea, affected capacity for orientation and return to the colony, metabolism disorders, altered pheromones level directly affecting behavior in the colony, lower immune capacity for defense and finally depopulated bee colonies (7). The presence of parasites is to be found in the entire bee colony in the structure of bees' intestine, inside the hive, in the hive products (honey), where they may persist beyond the clinical phase (7). The paper aims for a correlation between the existence of the spores in honey, the presence of infections in the bee colonies in the initial apiary and honey quality. The correlation may be directly between the degree of spore infestation and the main organoleptic and physical-chemical characteristics of honey. Our observations belong strictly to the field of research without any statistical and epidemiological evaluation of official implication.

Material and method

The research has been conducted in cooperation with private beekeepers that called on the Pathology Laboratory to establish the diagnosis during the active season period (2014-2015). A number of 65 canola honey samples have been examined out of which 26 samples were selected that had a positive diagnosis (presence of spores in honey) as well as a positive diagnosis of disease in the bee colony. (Fig. 1) The diagnosis of nosemoses disease was

established on the samples received from the owner by use of the standard method OIE 2008 (4).

Fig. 1. Canola Honey (canola pollen in the honey)

The honey was examined to diagnose the presence of spores and to quantify their number. Samples of 10 ml were collected in depth from three different areas in the whole volume of honey. They were homogenized and examined with the x 40 optic microscope to diagnose and count spores. The tested honey lots were classified into 3 groups in correlation with the infestation degree: group 1 (1-4 spores) (Fig. 2), group 2 (5-7 spores) and group 3 (≥ 7 spores). (Fig. 3) To identify the spores, the morphological diagnosis criteria were used (hygiene quality indicator of the tested honey) (4).

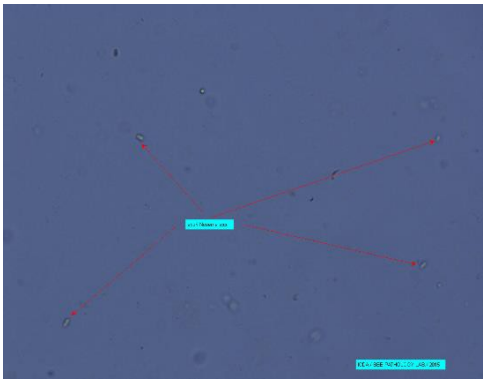

Fig. 2. Canola honey samples from group 1

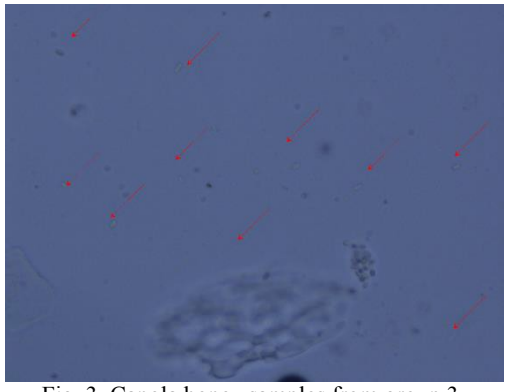

Fig. 3. Canola honey samples from group 3

For the organoleptic and physical-chemical analysis samples were sent to the Renar-accredited chemistry Laboratory and these were tested according to STAS standards and were evaluated according to criteria of organoleptic and physical-chemical quality (1, 2, 3, 9, 10). Sensory analysis as a scientific method to evaluate the organoleptic characteristics of honey has an important role in establishing its quality, being used not only to establish authenticity, classification and standardization but also to separate faulty samples and avoid contamination. Sensory analysis principles should be in conformity with the ones in STAS 784/3-1989 (1, 2, 3, 9. 10).

Fluid honey was examined organoleptically on samples as such, looking for foam and/or impurities, removing any contaminated samples with other agents that can alter honey. (Fig. 4, 5) After homogenization and filtering, parameters of aspect, consistency, color, smell and taste were also taken into account (1,2) Reference values had the following sensor

characteristics: foamless aspect, absence of foreign visible compounds, color from less to light yellow, golden-yellow, orange-yellow, dark yellow, reddish, brown yellow, dark brown, smell and taste typical of honey, more or less distinguished fragrance, sweet taste, homogenous, fluid, viscid, crystalized consistency (8).

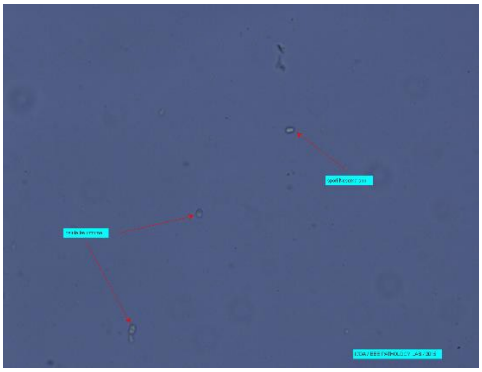

Fig. 4. Canola honey samples with *Nosema spp.* and yeast

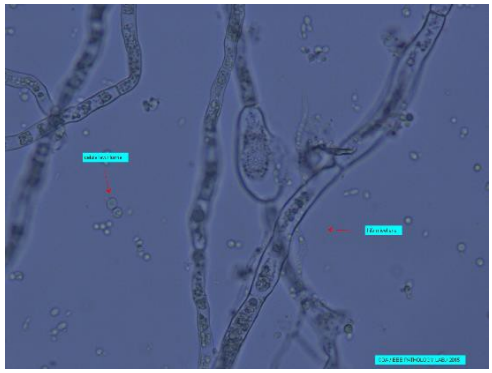

Fig. 5. Canola honey samples with *Nosema spp.* and fungal agents

Results and discussions

Microscopic method results are presented in table 1.

Table 1

Contamination degree of samples and distribution per groups

No. Sample	No. *Nosema spp.* spores /field	Group
1, 2, 3, 6, 9, 11, 12, 14,16, 18, 19,20, 21, 22, 23, 24, 26	1-4	1
4, 7, 8, 13, 15, 17, 25	5-7	2
5, 10	> 7	3

Depending on the contamination degree, the tested samples were classified into groups and it was noted that most samples belong to group 1, having a low contamination degree (1-4 spores/ microscopic field). In group 2 there are 7 samples, having a contamination degree of 5-7 spores/field, and group 3 displayed a high contamination degree only in 2 samples (≥ 7 spores/field).

The values of the witness lot were negative for the presence of *Nosema spp.* spores. Acidity did not exceed 4ºA having a water content of less than 20%, a pH of 3.5-4.5, and the HMF values under 2 mg /100 g.

The analysis of *physical-chemical* parameters for all 26 samples showed the following:

- ***Acidity*** was in the range of 1.3-4.1 ºA, the minimal excess of 4.1 ºA being noticed in samples 13 and 25, both having a medium infestation degree, as compared to the witness sample;
- ***Water content*** varied between 13-21.3 %, an excess of the admitted value (20%) being recorded in sample 13 of medium infestation degree, as compared to the witness sample;
- ***pH Value*** was of 3.8-4.2 no higher than the admitted maximum and minimum value having been found, as compared to the witness sample;
- The only altered parameter in the whole lot was that of the ***HMF (hydroximethilfurfural)*** value that registered increases to 2.07 mg/100 g and 10.7 mg/100 g, none of the samples

having the minimum accepted value for unprocessed honey of 1 mg/100 g honey, as compared to the witness sample (max 2 mg/100 g);

No correlation can be made between the groups distributed per *Nosema spp* spores contamination degrees and the changes in the physical-chemical parameters. However, 10 times higher HMF values were found in the samples contaminated with *Nosema spp* spores (2 samples).

The sensory analysis of the groups in the experiment presented low variations of the organoleptic parameters (consistency, aspect, transparency, color).

- Samples of high contamination degree (group 3) appeared opaque, having a medium or thick layer of foam, higher viscosity, humidity and mass crystallization (7,69 %);
- Samples of medium contamination (group 2) displayed mainly an altered yellow-brownish color, crystallization and opaque aspect with or without a foam layer (26,92 %);
- Organic and inorganic impurities were present in 10 cases without a direct correlation to the infestation degree (38,46 %).

Conclusions

The analysis of the *Nosema spp* spores infestation degree of honey showed in most cases a low infestation degree;

No positive correlation exists between the infestation degree of honey (hygienic quality) and the physical-chemical and organoleptic quality;

To two samples 10 times higher HMF values were found in the samples contaminated with *Nosema spp* spores;

The results that have been obtained showcase potential changes to the quality of honey in bee colonies infested with *Nosema spp.,* these being only preliminary results.

Acknowledgments

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