

THE USE OF BIOLOGICALLY ACTIVE SUBSTANCES FOR STRENGTHENING OF RESISTANCE TO DISEASES OF HONEYBEE COLONIES *APIS MELLIFERA*

Valentina Cebotari^{1*}, I. Toderas¹, I. Buzu¹

¹ Zoology Institute of Science Academy, Chișinău, Republic of Moldova

Abstract

The purpose of researches lies in cultivation and testing the bee food for some nutritional supplements (remedies) with high content of biologically active substances, in particular of proteins and microelements (Fe and Se), and working out the processes for bee families feeding in order to strengthen their resistance to overwinter and diseases. So, was cultivated the strain of cyanobacteria *Spirulina platensis* CNM-CB-02 in presence of selenite of Fe (III) hex hydrate - $FeSeO_3 \cdot 6H_2O$, given as part of nutritious environment in amount of 30 to 35 mg/L, first three days of cultivation. Subsequently, was obtained the extract from nominated biomass, by extracting of biologically active substances with an alcoholic solution of 20 to 30%, with sediment isolation from supernatant, drying of isolated sediment, double extracting with 0.45% NaOH solution of biologically active substances, combining of obtained supernatants and making of dialyze up to 7.5 – 8.5 pH, and finally obtaining the supplement, named by us "Apispir+Fe+Se". At the end of august 2010 and early April 2011, have been carried out comparative testing experiences of this supplement on bee colonies from three batches (table), within ten families in each batch. Test results have shown that feeding bees with nutritional supplement "Apispir+Fe+Se", at the end of august, during reduced harvest, has contributed to significantly higher overwintering resistance with 10.5 – 19.5% ($P < 0.001$). Feeding bees with nutritional supplement „Apispir+Fe+Se”, in the spring, at the beginning of April, during reduced harvest, has contributed to significantly higher disease resistance of bee colonies (measured by the test of hygiene speed and removal of dead brood from the comb cells), on the 15th day - with 8.4 – 18.5% ($P < 0.001$). Disease resistance remained higher at experimental groups also in June. Bee colonies from IIIrd experimental group, which have got in food nutritional supplement „Apispir+Fe+Se”, had a higher resistance to diseases, compared with IInd and Ist batches, with 7.9 to 15.5% ($P < 0.001$). The generalized result of using the nutritional supplement „Apispir+Fe+Se” consists in strengthening the bee immunity, conducting to their higher overwintering and disease resistance, also decreasing of their morbidity and mortality.

Key words: substances, biological, active, resistance, diseases, overwintering, bees

INTRODUCTION

Problems of fostering of *Apis mellifera* bee colonies, in order to accelerate their rate of growth and to reduce their morbidity and mortality always were concern of biologist researchers and beekeepers from many countries, both within Apimondia and its beyond [7, 11, 12].

At different times of the year, bee colonies are attacked by a number of pathogens of microbial, viral, micotic and parasitic origins.

According to scientific reports, during the winter it is found, often, bee colonies loss of more than 50-60% from bee population entered in winter [3, 6]. In some countries, have been recorded cases of "bee collapse" – total disappearance of bees from nest, caused by factors not identified with precision until now [4].

Naturally, the immune system of bees opposes resistance to pathogens. Bee colony has a native instinct of hygiene, expressed through removal from the nest of dead bees and larvae, isolation and removal of unknown bodies and impurities.

Speed of dead larvae's removal from the combs correlates directly proportional to bee

*Corresponding author: valentinaceb@yahoo.com
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colonies disease resistance, which is determined by the immune system and influenced by environmental factors, such as: harvest and food quality (as natural as additional), measures of care and disease prevention etc.

To strengthen disease resistance of bee colonies, beekeepers apply different procedures and means of feeding bees with nutritional supplements that contain, along with energo-proteic components, biologically active substances that stimulate vital functions of bees, as a result contribute to increase their immunity.

It is known a process for stimulating the physiological functions of bees and their protection against infectious diseases, which consists in feeding them for 30 days, with a mixture of sugar syrup and suspension of *Bacillus subtilis* 11B bacterium, which has a high level of enzyme activity and a broad spectrum of antibacterial action [11].

The drawback of this method is that the period of this supplements application is too long, and the use of the strain of *Bacillus subtilis* 11B bacterium, as a medicinal product applied for human medicine and, at the same time, for beekeeping, leads to increasing tolerance of micro-organisms towards this medicine and decreasing of its therapeutic effect, same as antibiotics.

According to these findings, researchers of Zoology Institute, together with researchers of Microbiology and Biotechnology Institute, from Science Academy of Moldova, have developed technologies for production of bioactive preparations of a new-generation compounds Apispir and BioRSp, with the primary source of biomass - Spiruline. Besides stimulating effect on queens prolificacy and bee colonies productivity, these preparations assume to strengthen the immune status of honey bees, thus contributing to overcoming the impact caused by the collapse of bee families (CCD) [1, 8, 9, 10].

The purpose of researches is to cultivate and test in bees food, the nutritional supplements (remedies) with high content of biologically active substances, in particular of protein and microelements (Fe and Se) and work out the processes for bees feeding in

order to strengthen their immunity and their resistance to overwintering and diseases. The purpose of researches is to cultivate and test in bees food, the nutritional supplements (remedies) with high content of biologically active substances, in particular of protein and microelements (Fe and Se) and work out the processes for bees feeding in order to strengthen their immunity and their resistance to overwintering and diseases.

MATERIAL AND METHODS

In order to achieve this purpose, was cultivated the strain of cyanobacteria *Spirulina platensis* CNM-CB-02 with presence of selenite of Fe (III) hexahydrate- $\text{FeSeO}_3 \cdot 6\text{H}_2\text{O}$, administered as part of the nutritious environment, in amount of 30 - 35 mg/L, in first three days of cultivation. Subsequently, was obtained the extract from nominalised biomass, by extracting biologically active substances with an alcoholic solution of 20 - 30%, its shaking and spinning with separation of sediment of supernatant. After, the sediment was dried separately at a temperature of 40 - 45°C, extracted the solution of 0.45% NaOH in 60 min at shaking, spinning, separation of sediment, repeated extraction of biologically active substances with 0.45% NaOH solution for 30 min at shaking, spinning, mixing of obtained supernatants and making of dialyze up to pH 7.5 - 8.5 with obtaining final supplement.

This supplement, called by us „Apispir+Fe+Se”, is a liquid green with yellowish shade, dry substance which contains 55 - 65% of protein, including the entire set of essential and non essential amino acids. The supplement contains, as component bioactive part, Fe (III), in amount of 0,8...1,0%, and 0,2...0,3% as some of the basic antioxidant, imunomodulator and fortifying elements of haemolymph circulatory system which represents immune system of the bee.

Experimental works for testing the remedy were carried out on bee colonies *Apis mellifera Carpatica* race, at the experimental apiary of Zoology Institute from Science Academy of Moldova.

To estimate the efficiency of nutritional supplement „Apispir+Fe+Se”, at the end of August of 2010 and early April 2011, have

been carried out comparative testing experiences on bee colonies from three batches (table), with ten families in each batch. Bee feeding was done with a mixture of extract from biomass of cyanobacteria *Spirulina platensis* CNM-CB-02 stalk with sugar syrup of 50% ratio of 1/500, respectively, in quantity of 100 - 130 ml of the mixture for a frame with bees, every 2 days, for a period of two weeks.

Bee colonies from Ist batch - witness, have received as food only sugar syrup without any biologically active substances, bees from IInd batch received as food, sugar syrup enriched with supplement II (Apispir), MD-3158 G2 2006. 10. 31, while the ones from the IIIrd batch – have received sugar syrup enriched with supplement „Apispir+Fe+Se”. At the spring examination in 2011, has been studied the overwintering resistance of bees, and in June of the same year, their resistance to diseases.

Determination of characters of overwintering and disease resistance has been carried out, in accordance with methodology developed by us [2] in the Zoological rule concerning bee colonies bonitation, growth and certification of beekeeping genitor materials, approved by Government Decision No. 306 of 28.04.2011 (M.O. nr. 78-81 of 13.05.2011, art. 366) [5].

Data from experiences have been processed in accordance with biometric variation statistic, according to the methods of Plohinschii N. A. 1969 [14].

RESULTS AND DISCUSSIONS

Test results have shown that feeding bees with nutritional supplement "Apispir+Fe+Se" at the end of august, during the period of reduced harvest, has contributed to significant increase in value of overwintering resistance character (table).

It was ascertained that overwintering resistance of bee colonies from IIIrd batch grew compared with IInd and Ist batch with 9.1 – 15.6 percent points, or 10.5 – 19.5%, respectively ($P < 0.001$).

This is explained by the fact that biologically active substances from nutritional supplement "Apispir+Fe+Se"

have helped to strengthen bees immunity and, as a result, increasing of overwintering resistance, what represents the amount of surviving bees over winter, determined by correlation of bees amount left over winter with bees amount at the beginning of winter, expressed in percentage.

At the same time, feeding bees with nutritional supplement „Apispir+Fe+Se”, in the spring, at beginning of April, during the period of reduced harvest, has contributed to significant increase of the value of disease resistance of bees (measured by the test of hygiene speed and removal of dead brood from comb cells).

It was ascertained that, on 15th day of starting to introduce in bees food of nutrient supplement „Apispir+Fe+Se”, bees resistance to disease in IIIrd batch increased significantly compared to Ist (witness) and IInd batches with 7,4 - 14,9 percent points, or 8.4 – 18.5%, respectively ($P < 0.001$).

Disease resistance remained even higher in July at the bees from experimental batches, which received in their food biologically active substances.

Therefore, hygienic test applied this time, has shown that bee colonies from IIIrd experimental batch, which received in their food, nutritive supplement „Apispir+Fe+Se”, have had a higher resistance to diseases, compared to bees from Ist and IInd batches with 7,2 - 13,1 percent points, or with 7.9 – 15.5 % ($P < 0.001$).

Generalized results of using nutritive supplement „Apispir+Fe+Se” consists of strengthening of bees immunity, followed by increasing of bees resistance to diseases and overwintering, and also decreasing of bees morbidity and mortality.

The obtained effect is determined by the presence of biologically active substances in nutritive supplement, as are: amino acids in big quantities, peptides, vitamins, pigments and micro elements, especially, Fe (III) and Se in big quantities, being catalysts of important functions of vital bee's activity, with great stimulating, immunomodulating and anti oxidant properties.

It is well known that vertebrates, has iron as a part of haemoglobin and myoglobin composition transported by blood

erythrocytes, as well as a number of enzymes that contribute to improvement of oxygen transportation to the texture cells, but selenium, as a component part of some

hormones and enzymes, involves in the process of erythrocytes recovering and stimulating immune system of the body [13].

Table 1 Values of characters of resistance to overwintering and diseases of bee colonies from experimental batches

Specification	Ist batch, witness (n = 10)		IInd batch, „Apispir” MD 3158 G2 2006.10.31 (n = 10)		IIIrd batch, invention „Apispir+Fe+Se” (n = 10)		
	Average value of character	%	Average value of character	% Compared to witness batch	Average value of character	% Compared to witness batch	% Compared to IInd batch
Resistance to overwintering, %	80.1±0.9	100	86.6±1.0	108.1	95.7±1.1	119.5	110.5
Resistance to diseases, %: At 15th day In June	80.4±1.0	100	87.9±0.9	109.3	95.3±1.2	118.5	108.4
	84.7±1.1	100	90.6±1.1	107.0	97.8±1.3	115.5	107.9

n* - number of bee colonies in batch

Unlike the blood of vertebrates, hemolimfa of bees does not contain the respiratory pigment (haemoglobin), therefore it is not taking part in breathing, but has an ultimate role in ensuring nutrition functions in organs and textures of the body, as well as those of body defending against pathogenic agents, having a huge amount of white cells of different forms and types. In hemolimfa consistence you'll find absolutely all necessary substances for bee metabolism, including: energy substances, protein, amino acids, enzymes, hormones, micro-macro-elements, including Fe and Se which determines immune system. The quality of hemolimfae and its saturation with nutritive necessary elements depends a lot on food and nutrition of the bee.

Therefore, biologically active substances taken by bees from nutritional supplement bee „Apispir+Fe+Se”, in period of reduced harvest, had a beneficial effect on the quality of hemolimfae composition, activating physiological immunomodulator functions of bees, which has led to immunity strengthening and increasing of resistance to diseases.

CONCLUSIONS

1. The cultivation of cyanobacteria *Spirulina platensis* CNM-CB-02 strain with

selenite of Fe (III) hexahydrate- $FeSeO_3 \cdot 6H_2O$, allows obtaining a nutritional supplement „Apispir+Fe+Se”, with increased content of biologically active substances.

2. Use of nutritional supplement „Apispir+Fe+Se”, in bees food in period of reduced harvest in nature, at the end of august, enhances resistance to overwintering of bee colonies, with 10.5–19.5% ($P < 0.001$).

3. Feeding bee colonies in period of reduced harvest in nature, at beginning of April, with nutritional supplement „Apispir+Fe+Se”, contributes to increasing of bee colonies resistance to diseases with 7.9–15.5% ($P < 0.001$).

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