THE ROLE OF "APISPIR + Zn" BIOSTIMULATOR IN INCREASING OF PRODUCTIVITY OF APIS MELLIFERA BEE COLONIES

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Abstract
The goal of this study was bio stimulator effect demonstration of nutritional supplements of sugar syrup enriched with extract stem biomass of cyanobacteria Spirulina platensis, grown on the base of monochloroacetic tetra hydrated zinc remedy, on morphological indices of Apis mellifera bee colonies. In bees feeding was tested an energy-mineral -protein nourishing supplement "Apispir+Zn", composed of sugar syrup in proportion 1:1, enriched with extract stem biomass of cyanobacteria Spirulina platensis, grown on base of coordination compound of monochloroacetic tetra hydrated zinc \( [\text{Zn(CH$_2$ClCOO)$_2$·12H$_2$O}] \), administrated in component of nutritive medium in amount of 30 ... 35 mg / L, in the first three days of cultivation. The extract stem biomass of cyanobacteria Spirulina platensis was obtained by repeated extraction of biologically active substances with alcoholic solution and NaOH solution, centrifugation, combination of obtained supernatants, and dialysis up to 7.5...8.5 pH with obtaining of final extract. Nutritional supplements "Apispir + Zn" was tested on two analogous batches, with 10 families in each batch, including: - Ith batch - control, whose bees received as food sugar syrup 1:1, in a quantity of 100 ... 130 mL for a frame with bees, every two days, for two weeks; - IIth batch - experimental bees, which received as food sugar syrup 1:1 in same quantity, enriched with supplement "Apispir + Zn" in relation to 500:1, respectively syrup and extract. In early May, before main harvest season, were studied the main morphological productive characters and features of bee colonies. The test results showed that feeding bees with nutritional supplement "Apispir + Zn" in early spring, during the poor harvest in nature, contributed to a significant increase of the value of the bee families main morphological and productive characters. Thus, bee colonies from experimental batch, which received as food nutritional supplement enriched with "Apispir + Zn", exceeded the other batch of bees: after queen prolificacy – with 607 eggs/24 hours, or with 32.5% (P<0.01), the amount of covered brood - with 73 hundred cells, or with 32.6% (P<0.01), colony strength – with 0.4 kg or 18.1% (P<0.01) resistance to disease - with 12.5 percentage points, or with 15.5% (P<0.001), flight intensity - with 2.0 bees / minute, or 20.8% (P<0.01), amount of honey gathered in the nest - with 1.54 kg, or 56.8% (P<0.001), the amount of bee bread gathered in the nest - with 20.0 hundred cells, or with 58.3% (P <0.01).

Key words: bio stimulator, „Apispir+Zn”, productivity, bee, colonies

INTRODUCTION
In Republic of Moldova, in poor periods for harvest in nature, especially, early spring (March-April) before the main picking, in bees food persists a deficiency of complete nutrients, such as protein, vitamins, trace elements, etc. In bees body of the bees is a deficiency of essential nutrients, which stagnates their vital activity and inhibits the subsequent processes of food accumulation in the nest.

An importance apart presents feeding the bees with micronutrients, as bioactive catalyst substances, which determines the activity of enzymes and serve as a substrate for cell regeneration in tissue of living organisms. Influence of microelements refers to nutrients digestion and assimilation processes. Particularly important are the trace elements for the transport of oxygen, regulating the hydrological regime in the body, neutralizing the dissimilation products as a result of oxidation processes.

Among trace elements, a significant role has the Zinc as biologically active substance.
It is contained essentially in honey, Royal Jelly, pasture and other bee products.

Minerals, including trace elements, get into the bees body through food as water, pollen, nectar. The presence of nutrients in these foods of bees determines, in large part, the vital activity of the organism, and their content in bee products.

In order to fill the deficit of biologically active substances, including nutrients, in bees food and unlocking of their vital processes during their activity during deficitary picking periods, beekeepers apply different procedures and means to stimulate vital functions of bees [7, 8, 9, 11, 12].

It is known the process of increase of bees productivity, which consists of feeding them with a nutritional mixture composed of sugar syrup, pollen substituent, set of amino acids, trace elements, vitamins and powdered sugar, distributed into the beehive during April-May, once in 12 days, for 36 days [7].

The disadvantages of this technique are that it is expensive, the components of the supplement are complicated, the processes of preparation and application of nutrient mixture are too long, and the trace elements introduced in the form of salts are hardly digestible for bees and easily oxidable, causing disturbances in their digestive tract.

The above problems can be solved in part through using in bees food nutritional supplement from sugar syrup 1:1 enriched with biomass extract of cyanobacteria stem *Spirulina platensis* CNB-CB-02 [1, 3, 4]. Biomass extract of this stem contains a wide spectrum of biologically active substances, but, at the same time, contains less protein and micronutrients, particularly, zinc.

In this context, the development and testing of new nutritional supplements to feed bees in deficient harvest period is an actual problem.

**MATERIAL AND METHOD**

It was developed and tested in bees food, an energy-mineral-protein nutritional supplement composed of sugar syrup in proportion of 1: 1, enriched with biomass extract of cyanobacteria *Spirulina platensis*, grown in the presence of coordinative compound of monochloracetate of tetrahydrated zinc [Zn(CH2ClCOO)2·12H2O], administered in component of nutritional environment, in amount of 30... 35 mg/L in first three days of cultivation, as a result, the biomass gained a high content of biologically active substances, including micronutrients, especially of Zn (II) [5].

The biomass extract of cyanobacteria *Spirulina platensis* stem has been obtained by extraction of biologically active substances with alcoholic solution of 20...30%, its shaking and centrifugation with separation of supernatant sediment. After this, was done the drying of sediment at temperature of 40...45, extraction with NaOH solution of 0,45% for 60 min at shaking, centrifugation, sediment separation, repeated extraction of biologically active substances with NaOH solution of 0,45% for 30 min at shaking, centrifugation, combining of obtained supernatants and making dialysis up to 7.5...8.5 pH with obtaining the final supplement.

This extract, called by us "Apispir + Zn", presents a liquid of green colour with a yellowish shade, whose dry substance contains 60...70% protein, produced by the entire set of essential and nonessential amino acids. The extract contains as bioactive component, zinc ions (II), bivalent in quantity of 0.3...0.5%, one of the major antioxidant and catalysts elements of bees vital activity.

In order to estimate the efficiency of the nutritional supplement "Apispir + Zn", during the poor harvest period (early April), have been done comparative testing experiences of it, on bee colonies from two similar batches, with 10 families in each batch, including:

- 1st batch - witness, where the bees were fed with sugar syrup 1: 1, in amount of 100...130 mL for a frame with bees, every 2 days, for two weeks.
- 2nd batch - experimental, which were fed with sugar syrup 1:1 in the same amount, enriched with the supplement "Apispir+Zn", in proportion of 500:1, respectively syrup and extract.

In early May, before harvest, were examined the main morph productive characters and features of bee colonies, according to zoo technic norm, regarding bee colonies evaluation, increasing and certification of beekeeping genitor material [2].
The obtained data in experiments were processed statistically using computer software "STATISTICS - 6" and evaluated their certainty, according to variational biometric statistic, after the methods of Плохинский Н.А. 1969 [10]

RESULTS AND DISCUSSIONS

Test results have shown that feeding bees with the nutritional supplement "Apispir+Zn" early spring, during the poor harvest in nature, contributed to the significant increase of the main morph productive characters value of the bee colonies (table).

Thus, if at the beginning of experiences, the queens prolificacy of bee colonies from both groups was at same level, then over a month, this feature at bee colonies from II\textsuperscript{nd} batch, who received in food the nutritional supplement enriched with "Apispir+Zn", has increased significantly, compared to the witness batch, with 607 eggs/24 hours, or with 32.5 % (P < 0,01).

Table Values of morph productive features of bee colonies from experimental batches

<table>
<thead>
<tr>
<th>Specification</th>
<th>Ist batch M₁ ± m₁</th>
<th>II batch M₂ ± m₂</th>
<th>M₂ – M₁</th>
<th>% Compared to Ist batch</th>
<th>td</th>
</tr>
</thead>
<tbody>
<tr>
<td>Queens prolificacy, eggs/24 hours</td>
<td>1868 ± 117</td>
<td>2475 ± 171</td>
<td>607</td>
<td>132,5</td>
<td>2,93**</td>
</tr>
<tr>
<td>Covered brood quantity, hundred cells</td>
<td>224,0 ± 14,1</td>
<td>297,0 ± 20,6</td>
<td>73</td>
<td>132,6</td>
<td>2,92**</td>
</tr>
<tr>
<td>Bee colonies strength, kg</td>
<td>2,21 ± 0,08</td>
<td>2,61 ± 0,08</td>
<td>0,40</td>
<td>118,1</td>
<td>3,54**</td>
</tr>
<tr>
<td>Resistance to disease, %</td>
<td>80,4 ± 1,0</td>
<td>92,9 ± 0,8</td>
<td>12,5</td>
<td>115,5</td>
<td>9,76**</td>
</tr>
<tr>
<td>Flight intensity, bees/minute</td>
<td>9,57 ± 0,49</td>
<td>11,57 ± 0,43</td>
<td>2,00</td>
<td>120,8</td>
<td>3,06**</td>
</tr>
<tr>
<td>Quantity of honey, kg</td>
<td>2,71 ± 0,08</td>
<td>4,25 ± 0,33</td>
<td>1,54</td>
<td>156,8</td>
<td>4,54**</td>
</tr>
<tr>
<td>Quantity of pasture, hundred cells</td>
<td>34,10 ± 4,25</td>
<td>54,00 ± 5,81</td>
<td>20,00</td>
<td>158,3</td>
<td>2,78**</td>
</tr>
</tbody>
</table>

Remark: ** B > 0, 99; *** B > 0,999

Such a growth regularity was observed also at covered brood quantity. It was established that this feature has increased at bee colonies from experimental batch, directly proportional to prolificacy, exceeding the values from witness batch with 73 hundreds of cells, or with 32,6% (P<0,01).

Increasing of breeding functions activity of bee colonies from II\textsuperscript{nd} batch, compared to witness batch, has led to increasing of working bees quantity in the nest. Thus, bee colonies from II\textsuperscript{nd} batch, which received in food nutritive supplement enriched with „Apispir+Zn”, exceeded significantly by strength, other bees from witness batch with 0,4 kg, or with 18,1 % (P < 0,01).

It was established that feeding bees with a nutritional supplement enriched with "Apispir+Zn" contributes to strengthening their resistance to disease, determined from the standard test of hygienic instinct of dead brood removal. Thus, disease resistance of bee colonies fed with the nutritional supplement enriched with "Apispir+Zn" increased compared to witness batch, with 12,5 percentual units, or with 15,5% (P<0,001).

Research results demonstrates that the nutritional supplement enriched with "Apispir+Zn", administered in bees food, exerts a general cumulative influence to their vital activity functions. It was found that worker bees from II\textsuperscript{nd} batch had a bigger flight activity, compared with bees from witness batch, on average with 2,0 bees/minute, or 20,8% (P<0,01).

Bee colonies morph productive performance from experimental batch, compared to witness batch, are reflected more obvious in the diagram (fig.). Zero line on abscissa means the level of witness batch.

Due to activation of physiological functions of the queen and working bees, bees from II\textsuperscript{nd} batch, who received the nutritional supplement enriched with
"Apispir+Zn", have accumulated larger volumes of production in the nest. Thus, the quantity of honey accumulated in nest by bee colonies from II\textsuperscript{nd} batch was significantly higher, compared to witness batch, with 1.54 kg, or 56.8\% (P<0.001).

Also, the amount of pasture accumulated in the nest by bee colonies from II\textsuperscript{nd} batch was higher, compared to bee colonies from witness batch, with 20.0 hundreds cells, or with 58.3\% (P<0.01).

Generalized result achieved as an outcome of feeding bee colonies with nutritional supplement enriched with "Apispir+Zn", is due to the presence in it, of biologically active substances, such as: increased quantity of amino acids, peptides, vitamins, pigments and trace, especially zinc in form of bivalent ions in big quantities, being the catalysts of important functions from bees vital activity, increased stimulating and antioxidant properties.

All this has led to intensification of bees physiologically vital functions, especially the queens prolificacy activity, bee colonies resistance to disease, flight activity of working bees, which contributed to increase the production volumes accumulated in the nest.

CONCLUSIONS

1. To fill the deficit of complete nutrients by feeding the bees in periods of poor harvest in nature, can be used a nutritional supplement made from sugar syrup in a proportion of 1:1, enriched with "Apispir+Zn" (Patent MD 475 Z 2012.09.30.).

2. The biologically active substances in the nutritive supplement "Apispir+Zn", especially bivalent zinc (II), contribute to bees vital activity stimulation, accelerating the speed of brood breeding, increasing of bee colonies strength, their resistance to disease and ability to accumulate production in the nest.
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