TESTING OF THE NUTRIENT SUPPLEMENT ENRICHED WITH BIOMASS OF AQUATIC ALGAE *SCENEDESMUS APICULATUS* IN THE BEE'S FEED

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Abstract

The purpose of this study was to test in the bee's feed the biomass of aquatic microalgae Scenedesmus apiculatus, hereinafter referred to as bioactive supplement "Scenecuadri" and elaboration on its basis of a process of feeding of bee families during the end of winter and start of spring (february-march), poor harvesting period in nature. The researches were conducted on the Apis mellifera Carpatica bee families at the experimental apiary of the Institute of Zoology of the Academy of Sciences. For testing of biomass in bee's feed at the end of February, they were formed three batches of bees families, to which once for each frame with bees were administered 200 g of nutritional paste, prepared by mixing the powdered sugar with honey in proportion 7:3. The batch I - control, bees have received only nutritional paste, prepared by mixing the powdered sugar with honey. The batch II - the bees have received paste enriched with nutritional supplement „Apispir+Zn" in quantity of 200 mg of active substance per 1 kg of paste. The batch III - bees have received nutritional paste enriched with bioactive supplement „Apiculatus" in a quantity equivalent to 200 mg of dry substance per 1 kg of paste. Research results have shown that feeding of bee families with nutritional supplement enriched with biomass of aquatic microalgae S. apiculatus help to increase, compared to the control batch, queen prolificacy up to 164 eggs or 10,3% (t_d=5.2; P<0.001), the amount of capped brood with 17.8 hundreds cells or 9.3% (t_d=4.7; P<0.001), family power with 0.23 kg or 7.1% (t_d=3.0; P<0.01), resistance to disease by 3.4 points or 3.8% (t_d=3.6; P<0.01), brood viability with 1.2 points or 1.3% (t_d=2.9; P<0.01) the amount of bee bread accumulated in nest with 24.9 hundreds cells or 27.6% (t_d=8.4; P<0.001), the amount of wax increased by 0.11 kg or 36.7% (t_d=5.0; P<0.001) and the amount of honey at first harvesting with 4.18 kg or 38.9% (t_d=9.9; P<0.001). The result is due to increasing nutrient assimilation and accessibility of biomass, given the fact that the micro S. apiculatus is covered with a thin protective membrane and the biomass is rich in biologically active substances, in particular proteins, carbohydrates, lipids, essential amino acids, micro - and macro elements, antioxidants (β-carotene), which have a catalytic role in the metabolism of substances nitrogenous to worker bees, participates in the synthesis of enzymes, improves the vital activity of bees, with direct influences on their flight intensity, activating nectar and pollen harvesting functions, as well as secretory functions of wax glands, stimulates the functions of reproductive system of the queen, activating ovogenesis and egg-laying. All of this mostly determines the power of bee families, their capacity to accumulate precursors of beekeepers' products (nectar, pollen) and their productive potential as a whole.

Key words: bees, nutritional supplement, biomass, microalgae, *Scenedesmus apiculatus*

INTRODUCTION

The problem of honeybee nutrition during periods of poor harvesting in nature always remains topical for beekeepers, professionals and researchers in the field. At the end of winter and early spring (March), the reserves of natural food in the nest of the bee family are exhausted and there is a deficiency of bioactive nutrients in the bee organism, especially of carbohydrates, proteins, micronutrients, vitamins, which plays a decisive role in the physiological processes of the vital activity of the bees, determining the reproductive capacity and the future development of the bee families as a whole [3, 4, 5, 6, 7, 8, 20].

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In order to compensate the lack of nutrients in bee nourishment during of poor harvesting in nature, most beekeepers feed bees with sugar syrup, which is poor, excluding carbohydrates, in biologically active substances. Besides, during winter (February) or early spring (March), when the danger of frosts persists and the bees are in winter cluster, the use of sugar syrup is problematic. Under these circumstances, identification of accessible sources of biologically active substances and finding appropriate forms of distribution, to enrich nutritional supplements in bee ration during periods of inadequate harvesting in nature, is a current problem.

During the last decades a number of biologists have noticed the biomass of mono- or pluricelular microalgae as important sources of biologically active substances. Among these, most studied became the microalga *Chlorella vulgaris* and pluricelular microalga (cyanobacteria) *Spirulina platensis* [9, 10, 11, 15, 30, 31]. Research has shown that the biomass of microalgae *Chlorella vulgaris* contains an important set of biologically active substances. According to some authors [10], *Chlorella* is called the "*supplement of energy and vitality*", having therapeutic properties by improving the health of the body in general, and fortifing the immune system in particular, it's increase body resistance to infections. This microalgae, rich in β-carotene, is capable to remove toxic residues, including pesticides, from body ingested by food, extract the mercury deposits, and therefore is a powerful detoxicant. Administration of biomass suspension of this microalgae in bees food has contributed to the growth of colonies by 17.0-22.4% [8].

Spirulina platensis, among the many pluricellular algae species, has been studied most [1, 2, 13, 14, 15, 16, 17]. During several decades this filamentous pluricelular cyanophilic microalga was explored as a food source. The World Health Organization and the 3rd International Congress of Food Sciences and Technologies have unconventionally defined *Spirulina* as an essential source of up to 50 bioactive substances, that ensures the normal development of vital processes in the human and animal body [15].

To strengthen the vigor and disease resistance of bee families, some of researches have proposed to enrich the nutritional supplements with biomass of strain *Bifidocbacterium globosum* + biomass of *Streptococcus faecium* + carbohydrate + oxide and aluminum hydroxide + ascorbic acid [33], suspension the *Karnitinhlorid* [32], and strains of *Micromycetes* [18].

In beekeeping are known also other proceeding to stimulate growth of bee families by feeding with sources of biologically active substances, in particular sugar syrup mixture of 50% enriched with biomass microalgae *S. platensis (Nordst.) Geitl CALU-835* [19]. The disadvantage of this proceeding is the low efficiency, because the cells of that cyanophytes microalgae are covered with relatively thick a protective membrane, which stagnates the digestion process of nutrients from biomass by bees, in addition, the sugar syrup can not be used in winter when air temperatures are low.

Among the latest known new ways of enriching nutritional supplements with biologically active substances [21, 22, 23], the nearest solution after technical essence and the obtained result is the proceeding of feeding bee families *Apis mellifera* MD 475Z 2012.09.30 [21], which includes the feeding of bees in spring with a mixture of solution of 1% mas. biomass extract of microalgae *S. platensis* CNM-CB-02 and 50% sugar syrup in the ratio of 1: 500, respectively. Previously, to obtain biomass of *S. platensis,* microalgae has been cultivated in the presence of coordinative organic compound of *monochloracetate of Zn(II) tetrahydrate* – $Zn(CH_2ClCOO)_2 \bullet 4H_2O$, in concentration of 30 mg/l, which increases the permeability of living cells. The feeding of the bees with this mixture was carried out every 2 days, for two weeks in an amount of 100 ... 130 ml of a mixture on bee frame.

The disadvantage of this proceeding is the fact that the technology of obtaining of the extract biomass of microalgae *S. platensis* cultivated in the presence of coordinative compound is too complicated and expensive, and the mixture of sugar syrup enriched with supplement of bioactive substances can not be used during winter or early spring,

because of high humidity created by a large amount of releasing vapor in the nest during the period when bees are in hibernation skein. For these reasons, some researchers [24, 25, 29] have proposed as a source of biologically active substances – biomass of aquatic microalgae, which are more accessible and less expensive.

In this context, the aim of this paper was to test in feeding of bees biomass of aquatic microalgae *Scenedesmus apiculatus* and elaboration on its basis of a proceeding for feeding of bee families during the end of winter and start of spring (february-march), poor harvesting period in nature.

MATERIALS AND METHODS

The researches were conducted on the *Apis mellifera Carpatica* bee families at the experimental apiary of the Institute of Zoology of the Academy of Sciences of Moldova. To achieve the purpose, experimental plan has been made that included the feeding of bee families at the end of winter during poor harvesting in nature, when atmospheric temperatures were low, with the nutrition paste wich was prepared by mixing powdered sugar with honey in proportion 7:3 and bioactive supplement. As a bioactive supplement the biomass of aquatic microalgae *S.apiculatus,* in an amount of 10 ml/1 kg of the mixture, was used. The feeding of the bee families with enriched paste was performed by its distribution in the form of cakes in the nest, above the frame. Usually one cake for every frame with bees was put.

Bioactive supplement, called by us "*Apiculatus"*, represents a suspension of biomass of 2%, greenish yellow coloured, dry matter contains 38-48% proteins, which includes the complete set of essential and non-essential amino acids, 30-34.5% of carbohydrate, 10-11% of lipids, vitamins, micro- and macro elements and other important bioactive substances. In 100 mg of dry matter of the supplement there is 0.27-0.30 mg of β-carotene, one of the main components with antioxidant and catalytic properties on vital processes in working bees body. Considering that the monocellular microalgae *Scenedesmus apiculatus* var. *indicus* CNMN-AV-09 is covered by a relatively thin protective membrane, bioactive substances from biomass are available for digestion in the digestive tract of honey bees.

To estimate the efficiency the proceeding of the bees feeding with above-mentioned nutrient supplement, at the end of february were initiated experiments of comparative testing of the paste on bee colonies divided into three batches, 13 to 15 families each batch, according to the scheme in table 1.

Table 1 Scheme of the testing of nutritional supplements, enriched with biologically active substances from the biomass of aquatic algae, in bees' feed

Batch	No. of families/ batcn(N)	Name of the supplement and algae species, cultivated and introduced into the nutritional mixture	Amount and concentration of the solution per 1 kg paste	The amount of active substance per 1 kg of paste
I (control)	14	Energy paste – *without biomass*	-	-
II	15	Energy paste + *Apispir+Zn*	2 ml of 10%	200 mg
III	13	Energy paste + *Scenedesmus apiculatus*	10 ml of 2%	200 mg

The supplement was administered once, 200 g nutritional paste (a cake) for every frame with bees. The batch I - control, bees have received only nutritional paste, prepared by mixing the powdered sugar with honey in previously mentioned proportion. The batch II – the closest solution, the bees have received paste enriched with nutritional supplement "*Apispir + Zn*"[21] in quantity of 2 ml solution with a concentration of 10% (200 mg of active substance) per 1 kg of paste. The batch III - the proposed invention, bees have received nutritional paste enriched with bioactive supplement "*Apiculatus*" in a quantity 10 ml of suspension with a concentration of 2.0% (200 mg of dry substance) per 1 kg of paste.

In 100 days after feeding bees with the nutritional bioactive supplement (which

coincided with the first harvest) principal morph-productive characters of reproduction, development and productivity of bee families in the experimental batches were evaluated, according to Zootechnical norme regarding breeding of bee families, the growth and certification of genitor beekeeping material, approved by Government Decision no. 306 of 28.04.2011 [12]. The obtained in experience data were statistically processed using computer software "STATISTICA - 6" and evaluated their certainty, according to variation biometric statistics, by methods of Plohinskiy N. A.(1969) [34].

RESULTS AND DISCUSSIONS

The test results showed that the feeding of bees with the supplement "*Apiculatus*" at the end of winter, during poor harvest in nature, has contributed to a significant increase of values of principale morpho-productive characters. (Tab. 2).

Table 2 The test results of feeding of bees families with nutritional supplements fortified with algal biomass

Batch and Name of supplement	Nr. of bee fam.	The value of the character at first harvesting M ± m	The difference compared to batch I (control)			The difference compared to batch II (the nearest solution)		
			d	%	td	d	%	t_d
Prolificacy of queen, eggs/24 hours								
I control	14	1593 ± 25	-	-	-	-25	1.5	0.7
II Apispir+Zn	15	1618 ± 26	+25	1.6	0.7	-	-	-
III Apiculatus	13	1757 ± 19	+164	10.3	5.2***	+139	8.6	4.3***
Quantity of capped brood, hundred cells								
I control	14	191.5 ± 3.1	-	-	-	-2.7	1.4	0.6
II Apispir+Zn	15	194.2 ± 3.2	+2.7	1.4	0.6	-	-	-
III Apiculatus	13	209.3 ± 2.2	+17.8	9.3	4.7***	+15.1	7.8	3.9***
Family strength, kg								
I control	14	3.22 ± 0.05	-	-	-	-0.03	0.9	0.5
II Apispir+Zn	15	3.25 ± 0.04	+0.03	0.9	0.5	-	-	-
III Apiculatus	13	3.45 ± 0.06	+0.23	7.1	3.0**	+0.20	6.1	2.9**
Resistance to disease, %								
I control	14	88.9 ± 0.8	-	-	-	-1.5	1.7	1.6
II Apispir+Zn	15	90.4 ± 0.5	+1.5	1.7	1.6	-	-	-
III Apiculatus	13	92.3 ± 0.5	+3.4	3.8	3.6**	+1.9	2.1	2.7*
Broods viability, %								
I control	14	90.0 ± 0.3	-	-	-	-1.1	1.2	3.1**
II Apispir+Zn	15	91.1 ± 0.2	+1.1	1.2	3.1**	-	-	-
III Apiculatus	13	91.2 ± 0.3	+1.2	1.3	2.9**	+0.1	0.1	0.3
Quantity of wax, kg								
I control	14	0.30 ± 0.01	-	-	-	-0.01	3.3	0.7
II Apispir+Zn	15	0.31 ± 0.01	+0.01	3.3	0.7	-	-	-
III Apiculatus	13	0.41 ± 0.02	+0.11	36.7	5.0***	+0.10	9.7	5.0***
Quantity of bee bread, hundreds of cells								
I control	14	90.1 ± 2.1	-	-	-	-7.6	7.8	2.5*
II Apispir+Zn	15	97.7 ± 2.1	+7.6	8.4	2.6*	-	-	-
III Apiculatus	13	115.0 ± 2.1	+24.9	27.6	8.4**	+17.3	17.7	5.8***
Quantity of honey, kg								
I control	14	10.74 ± 0.35	-	-	-	-1.03	9.6	2.0*
II Apispir+Zn	15	11.77 ± 0.37	+1.03	9.6	2.0*	-	-	-
III Apiculatus	13	14.92 ± 0.24	+4.18	38.9	9.9***	+3.13	26.8	7.3***

Remark*: * P<0.05; ** P<0.01; *** P<0.001.*

It was found that the biologically active substances of supplement "*Apiculatus*" indirectly have caused stimulation of reproductive functions of the queen (oogenesis),

contributing to the increasing of egg-laying and number of capped brood in the nest, as a result, to the development of the bee family.

Because the queen does not consume nutritional supplement, administered in the nest, but it is constantly fed by worker bees with royal jelly, we can say that the biologically active substances in the supplement had a stimulating impact on lactogenic functions of the bee-nurse and on the qualitative composition of royal jelly, stimulating, thus, reproductive functions of the queen. As a result, the queen's prolificacy in hives from the batch III increased significantly compared with those in batch I (control) and batch II (the nearest solution), respectively, with 164 and 139 eggs / 24 hours, or 10.3 and 8.6% (td = 5.2 and 4.3; P<0.001).

Also, the quantity of capped brood at the hives from the batch III was significantly higher compared to batch I and batch II, 17.8 and 15.1 hundred cells, or 9.3 and 7.8% (td = 4.7 and 3.9, P<0.001), respectively.

The increasing of the queens prolificacy and amount of capped brood indirectly led to a significant increase of family strength, expressed by the total population of bees in the nest. Thus, the power of bee families from the batch III was significantly higher compared to group I and group II, 0.23 and 0.20 kg of bee, or 7.1 and 6.1% (td = 3.0 and 2.9; P < 0.01), respectively.

Test results have shown that feeding of bees with nutritional supplement "*Apiculatus*" during the poor harvesting period in nature, has contributed to the significant increase of values of the disease resistance and viability characteristics of bee brood. It has been found that biologically active substances from nutritional supplement "*Apiculatus*" have a stimulating impact on immune modulating functions, accelerating the hygienic instinct of bees, contributing to the strengthening of disease resistance. Thus, the biologically active substances from microalgal biomass had beneficial influence on properties of disease resistance and brood viability. As a result, 100 days after feeding with the nutritional supplement, the resistance of the bee families in the III batch of bees increased significantly compared to the I batch and batch II, with 3.4 and 1.9 absolute points, or 3.8 and 2.1% (t_d=3.6 and 2.7; P<0.01 and P<0.05).

Due to the action of the biologically active substances from nutritional supplement, working bees in the experimental families had more intense vital activity, indirectly contributing to the increase of the bee brood viability by feeding its with royal jelly, honey and bee bread.

So the brood viability of bee families of batches II and III, was significantly higher compared with controls, with 1.1 and 1.2 absolute points, or 1.2 and 1.3% (t_d=3.1 and 2.9; P<0.01). Given that the variability of these biological features (disease resistance and brood viability) is very narrow, the significance of these differences (small, at first sight, as absolute dimensions) is quite large and corresponds to a high degree of certainty, according to the probability theory of predictions, without errors after Student [34].

Despite of the fact, that the brood viability and resistance to diseases are biological characteristics largely determined hereditary (h^2 = 0.7-0.8), however, the test data demonstrates that feeding of bees with biologically active substances contribute, with certainty, to the strengthening of immunity and vigor of bee families, as a result - to the increase of their vital activity. Due to higher family strength, the colonies from experimental batches II and III have obtained more pronounced feature of accumulation of the final bee products in the nest, for which, actually, are bred and exploited the bees.

It was observed that the biologically active substances from nutritional supplement "*Apiculatus*" indirectly stimulated the vital activity, in particular the activity of flying of worker bees at first picking, thus contributing to the accumulation in nest of biger quantities of wax, bee bread and honey.

Thus, the amount of wax accumulated during this period in the nest was positively influenced by the nutritional supplement enriched with biomass of microalgae *S. apiculatus*. Bee families in the experimental batch III significantly exceeded the families from the control batch after amount of wax accumulated at the first harvesting with 0.11 kg or 36.7% (t_d=5.0; P<0.001) and families

from batch II with 0.10 kg or 9.7% (t_d=5.0; P<0.01).

The quantity of bee bread accumulated in the nest was also positively influenced by nutritional supplements enriched with algal biomass *S. apiculatus*. Thus, bee families from experimental batches II and III, who received nutritional supplements enriched both with extract of biomass of *Apispir+Zn* and biomass of microalgae *S. apiculatus* significantly exceeded the families from the control batch after the quantity of bee bread accumulated in the nest, respectively with 7.6 and 24.9 hundred cells, or 8.4 to 27.6% (t_d=2.6 and 8.4; P<0.05 and P<0.001). At the same time, the bee families in experimental batch III, compared to batch II -which received the food supplement enriched with Apispir + Zn, showed a higher bee bread accumulation in the nest, with 17.3 hundred cells or 17.7% (t_d = 5.8; P < 0.001).

Finally, the amount of honey accumulated in the nest, the morpho-productive character with important economic value, was also the most positively influenced by the biologically active substances contained in supplements enriched with both extract of biomass of *„Apispir+Zn"* and biomass of *S. apiculatus.* Thus after the amount of honey, collected at the first harvesting, the bees families from experimental batches II and III vastly exceeded families in the control batch I with 1.03 and 4.18 kg, or 9.6 and 38.9% (t_d=2.0 and 9.9; P<0.05 and P<0.001). The data presented in table reveals that bees families from the batch III had greatest capacity of accumulation of products in the nest, and after honey production, significantly exceeded the families from the experimental group II, with 3.13 kg, or 26.8%. This difference is veridical with the highest threshold of certainty without error probability forecasts after Stiudent (t_d = 7.3; P<0.001).

More obvious influence of biologically active substances from the extract or biomass of microalgae on the morpho-productive characters of bee families is presented in histogram 1.

In the histogram it can be seen that the characters of accumulation in the nest of bee products such as bee bread, wax and honey had the greatest growth rates. In particular the quantity of honey had rose the most obvious at the bee families who received by food biologically active substances from supplements enriched with biomass of aquatic microalgae *S. apiculatus.*

Technical result of the use of biomass of microalgae in the nutrition of bees consist in stimulate functions of ovogenesis and egg laying at queens, increasing the quantity of capped brood and of the number of hatched worker bees, which in its turn, led to the quantitative increasing of the power of bee families, the harbinger of higher productivity.

The result is due to increasing nutrient assimilation and accessibility of biomass, given the fact that the micro S. quadricauda is covered with a thin protective membrane and the biomass is rich in biologically active substances, in particular proteins, carbohydrates, lipids, essential amino acids, micro and macro elements, antioxidants (β-carotene), which have a catalytic role in the metabolism of substances nitrogenous to worker bees, participates in the synthesis of enzymes, improves the vital activity of bees, with direct influences on their flight intensity, activating nectar and pollen harvesting functions, as well as secretory functions of wax glands, stimulates the functions of reproductive system of the queen, activating ovogenesis and egg-laying.

All this mostly determines the stimulation of all the physiological processes of the body of all members of the bee family, starting from the queen - the founder of social community (nest) and finishing with the working bees, the vitality of which defines the power of bee families, their capacity to accumulate precursors of beekeepers' products (nectar, pollen) and their productive potential as a whole.

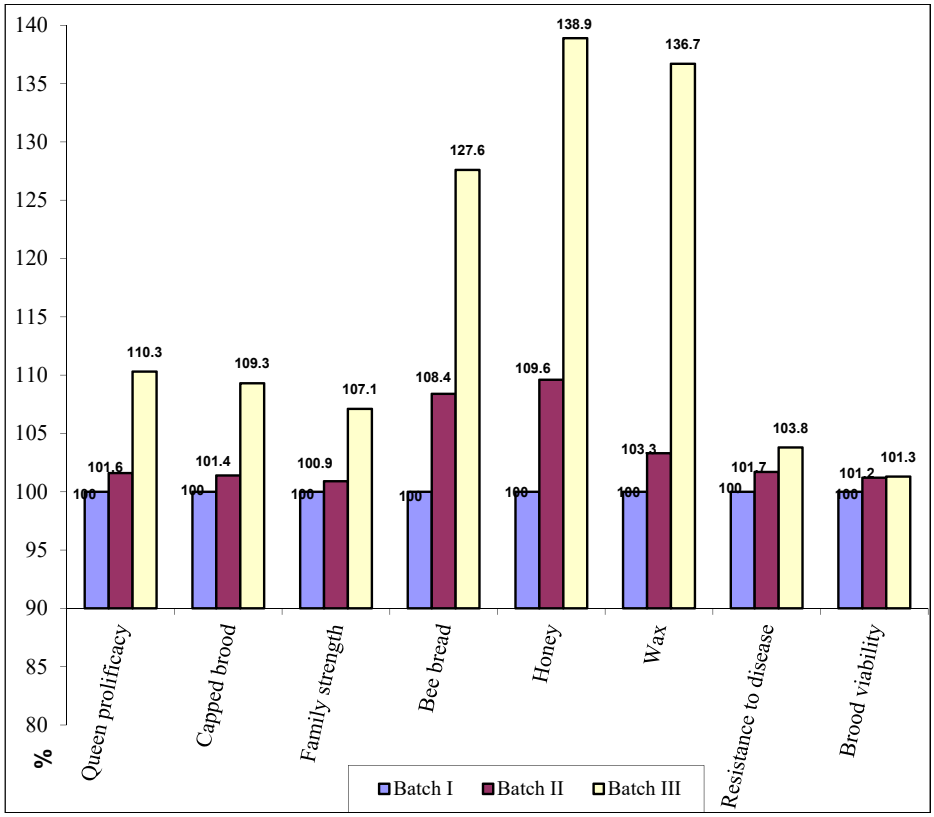

Fig. 1 Value of morpho-productive characters of bee colonies depending on the administered supplement

On the base the carried investigations it was elaborated a new proceeding of feeding of bee families [26] in poor harvesting period in nature, at the end of winter - early spring (February-March), when atmospheric temperatures are low. The proceeding provides the enrichment of the nutritional supplements with biologically active substances, more accessible and more easily digested by bees, which are a little cheaper and easier to obtain, ensuring at the same time, the nutritional needs of bees during this period of year. Biologically active substances, added to the food, stimulates prolificacy of bees queen, increase the power and productivity of bee families of *Apis mellifera.*

CONCLUSIONS

1. The feeding of bee families with nutritional supplement enriched with biomass of aquatic microalgae *S. apiculatus* help to increase, compared to the control batch, the queen prolificacy with 10.3% ($P<0.001$), the amount of capped brood with 9.3% ($P<0.001$), family power with 7.1% ($P<0.01$), resistance to disease with 3.8% ($P<0.01$), brood viability with 1.3% ($P<0.01$) and the amount of honey in the harvestig with 38.9% ($P<0.001$).

2. On the base the carried investigations it was elaborated a new proceeding of feeding of bee families (MD 1079 Y 2016.10.31) in poor harvesting period in nature, when atmospheric temperatures are low. This proceeding ensuring the increase of productivity of bee families [26].

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REFERENCES

[1]. Bulimaga Valentina, Rudic V., Zosim Liliana, Chiriac Tatiana, Turtă C., Prodius D., Şova S., Mereacre V. Procedeu de obţinere a biomasei de *Spirulina platensis*. Brevet de invenţie nr. MD 3128 G2 2006.08.31, Chişinău, 2006a.

[2]. Bulimaga Valentina, Rudic V., Zosim Liliana, Chiriac Tatiana, Turtă C., Prodius D., Melnic Silvia, Mereacre V. Procedeu de obţinere a biomasei cianobacteriei *Spirulina platensis*. Brevet de invenţie nr. MD 3129 G2 2006.08.31, Chişinău, 2006b.

[3]. Cebotari Valentina, Toderaş I., Buzu I. The use of biologically active substances for strengthening of rezistance to diseases of honeybee colonies *Apis mellifera*. University of Agricultural Sciences and Veterinary Medicine, Iasi, Romania. International Scientific Symposium: Modern zootehny, factor of sustainable development – Centennial 1912 - 2012. Lucrări Ştiinţifice, Cotaţie CNCSIS cu B$^+$, vol. 57, Seria Zootehnie, ISSN 1454-7368, Iaşi, 2012, pag. 39-43.

[4]. Cebotari Valentina, Toderaş I., Buzu I., Rudic V. The role of „Apispir+Zn" biostimulator in increasing of productivity of *Apis mellifera* bee colonies. University of Agricultural Sciences and Veterinary Medicine Iasi. Scientific Papers. Series Animal Science. Vol. 59, Impact CNCSIS B$^+$, ISSN 2284-6964; ISSN-L 1454-7368. Iasi, 2013a, p. 103-107..

[5]. Cebotari Valentina, Toderaş I., Buzu I., Rudic V. Use of chrome trace for vital activities functions stimulations of *Apis mellifera* bee colonies. University of Agronomic Sciences and Veterinary Medicine of Bucharest. Scientific Papers. Series D. Animal Science. Vol. LVI, Impact CNCSIS B$^+$, code index 1020, ISSN 2285-5750, ISSN-L 2285-5750. Bucharest, 2013b, p. 73-77.

[6]. Cebotari Valentina, Buzu I., Gliga Olesea, Postolachi Olga. New nutritional supplements for bees during deficient harvesting perionds. In: International Scientific Symposium „Modern animal husbandry – food safety and durable development" at the Univrsity of Agricultural Sciences and Veterinary Medicine Iasi. Scientific papers. Animal Science. Ed. „Ion Ionescu de la Brad". CD-Rom, Iaşi, 2016, p. 100-107, ISSN 2284-6964.

[7]. Cebotari Valentina, Buzu I., Postolachi Olga, Gliga Olesea. Testing of the nutrient supplement enriched with biomass of aquatic algaie in the bees feed. In: International Conference „Agriculture for Life, Life for Agriculturer" at the *University of Agronomic Sciences and Veterinary Medicine of Bucharest. Scientific papers. Series D. Animal Science*. Ed. „CERES" Publ. House. Vol. LIX, Bucharest, 2016, p. 85-90. ISSN 2285-5750.

[8]. Eremia N., Bahcivanji M., Zagareanu A. Study of algal *Chlorella vulgaris* suspencion on grouth and productivity of bees families. University of Agricultural Sciences and Veterinary Medicine Iasi. Scientific Papers. Animal Sciences. Vol. 59 (18), Iaşi, 2013, p. 148-152.

[9]. Ionov V.A., Basova M.M. Use of blue-green micro-seaweed Spirulina platensis for the correction of lipid haemostatic disturbances in patients with ischemic heart disease. Вопросы питания, 2003, 72:28-31.

[10]. Luca C. Chlorella. Sănătate cu dr. Cătălin Luca. Copyringht, 2012. http://dr.catălin.luca.ro.

[11]. Mazo V.K., Gmoshinskii I.V., Zilova I.S. Microalge Spirulina in human nutrition. Вопросы питания. 2004; 73:45-53.

[12]. Normă zootehnică privind bonitarea familiilor de albine, creşterea şi certificarea materialului genitor apicol, aprobată prin Hotărârea Guvernului nr. 306 din 28.04.2011 (M.O. nr. 78-81 din 13.05.2011, art. 366), Chişinău, 2011.

[13]. Rudic V., Zosim Liliana, Bulimaga Valentina, Chiriac Tatiana, Ciumac Daniela, Turtă C., Prodius D. Procedeu de obţinere a biomasei de *Spirulina platensis*. Brevet de invenţie nr. MD 3101 G2 2006.07.31, Chişinău, 2006a.

[14]. Rudic V., Bulimaga Valentina, Djur Svetlana. Mediu nutritiv pentru cultivarea cianobacteriei *Spirulina platensis*. Brevet de invenţie nr. MD 3417 G2 2007.10.31, Chişinău, 2007.

[15]. Rudic V., Bulimaga Valentina, Chiriac Tatiana et al. New nutraceuticals from *Spirulina*. International Conference on Exploatation of agricultural and food industry by-products and waste material trough the application of modern processing techniques. Institute of Bioengineer. and Environmental Protection–S.C. BIOING S.A., Bucharest, 2008a, p.26-29.

[16]. Rudic V., Toderas I., Gudumac V. et al. New remedies for bees. Akademos, Chişinău, 2008b, 4(11), p. 81-83.

[17]. Rudic V., Chiriac Tatiana, Bulimaga Valentina, Zosim Liliana, Gulea A., Poirier D., Ciapurina Ludmila, Ciumac Daniela, Toderaş I. Procedeu de obţinere a biomasei cianobacteriei *Spirulina platensis*. Brevet de invenţie nr. MD 3171 G2 2006.10.31, Chişinău, 2006b.

[18]. Starciuc N., Sârbu T., Bugneac V., Burţeva S., Postolachi O., Cebotari V. Soil micromicetes

antagonism against some pathogenes of *Apis mellifera*. The 12th International Symposium „Prospects for the 3rd Millenium Agriculture", Universitatea de Ştiinţe Agricole şi Medicină Veterinară. Book of abstracts. Section 9: Veterinary Medicine – Clinical sciences, Cluj-Napoca, România, 2013, p. 246

[19]. Toderaş I., Rudic V., Derjanschi V., Roman R., Gudumac V., Chiriac Tatiana, Bogdan V., Sicora Diana. Procedeu de stimulare a creşterii familiilor de albine şi sporirea productivităţii lor. Brevet de invenţie MD 2061 G2 2003.07.31, Chişinău, 2003.

[20]. Toderaş I, Rudic V., Gulea A., Cebotari Valentina, Buzu I. Influenţa remediilor organice bioactive de generaţie nouă asupra activităţii vitale a familiilor de albine *Apis mellifera*. Buletinul Academiei de Ştiinţe a Moldovei. Ştiinţele Vieţii, ISSN 1857-064X, categoria B, Nr. 3 (324), Chişinău, 2014, p. 4-15.

[21]. Toderaş I., Rudic V., Cebotari Valentina, Bogdan V., Gulea A., Bulimaga Valentina, Buzu I., Chiriac Tatiana, Arcan Elena. Procedeu de hrănire a familiilor de albine Apis mellifera. Brevet de invenţie MD 475 Z 2012.09.30. Buletinul Oficial de Proprietate Intelectuală, nr. 2, Chişinău, 2012a, p.28.

[22]. Toderaş I., Rudic V., Cebotari Valentina, Bogdan V., Gulea A., Bulimaga Valentina, Buzu I., Chiriac Tatiana, Railean Nadejda. Procedeu de hrănire a familiilor de albine Apis mellifera. Brevet de invenţie MD 476Z 2012.09.30. Buletinul Oficial de Proprietate Intelectuală, nr.2, Chişinău, 2012b, p.28-29.

[23]. Toderaş I., Rudic V., Cebotari Valentina, Bogdan V., Gulea A., Bulimaga Valentina, Buzu I., Chiriac Tatiana, Silistrari A. Procedeu de hrănire a familiilor de albine Apis mellifera. Brevet de invenţie MD 477 Z 2012.09.30. Buletinul Oficial de Proprietate Intelectuală, nr. 2, Chişinău, 2012c, p. 29-30.

[24]. Toderaş Ion, Cebotari Valentina, Ungureanu Laurenţia, Buzu Ion, Gheorghiţă Cristina. Procedeu de hrănire a familiilor de albine *Apis mellifera*. Brevet de invenţie nr. MD 1061 Y 2016.08.31. Agenţia de Stat pentru Proprietatea Intelectuală a Republicii Moldova. Buletinul Oficial de Proprietate Intelectuală, nr. 8, Chişinău, 2016, p. 33-34.

[25]. Toderaş Ion, Cebotari Valentina, Ungureanu Laurenţia, Buzu Ion, Gheorghiţă Cristina. Procedeu de hrănire a familiilor de albine *Apis mellifera*. Brevet de invenţie nr. MD 1062 Y 2016.08.31. Agenţia de Stat pentru Proprietatea Intelectuală a Republicii Moldova. Buletinul Oficial de Proprietate Intelectuală, nr. 8, Chişinău, 2016, p. 34.

[26]. Toderaş Ion, Cebotari Valentina, Ungureanu Laurenţia, Buzu Ion, Gheorghiţă Cristina. Procedeu de hrănire a familiilor de albine *Apis mellifera*. Brevet de invenţie nr. MD 1079 Y 2016.10.31. Agenţia de Stat pentru Proprietatea Intelectuală a Republicii Moldova. Buletinul Oficial de Proprietate Intelectuală, nr.10, Chişinău, 2016, p. 29.

[27]. Toderaş I., Cebotari Valentina, Gulea A., Buzu I., et al. Procedeu de hrănire a familiilor de albine *Apis mellifera*. Brevet de invenţie nr. MD 850 Z 2015.08.31. Agenţia de Stat pentru Proprietatea Intelectuală a Republicii Moldova. Buletinul Oficial de Proprietate Intelectuală, nr. 12, Chişinău, 2014, p. 29-30.

[28]. Toderaş Ion, Cebotari valentina, Gulea Aurelian, Buzu Ion, Floquet Sebastien, Cadot Emmanuel. Procedeu de obţinere a compusului [tetraoxoetilendiaminotetraacetatdimolibden V] de bis-(tetrafenilfosfoniu) di-semihidrat şi procedeu de hrănire a albinelor cu utilizarea acestuia. Brevet de invenţie nr. MD 4438 B1 2016.10.31. Agenţia de Stat pentru Proprietatea Intelectuală a Republicii Moldova. Buletinul Oficial de Proprietate Intelectuală, nr. 10, Chişinău, 2016, p. 22-23.

[29]. Ungureanu Laurenţia, Gheorghiţă Cristina. Tulpina microalgei verzi *Scenedesmus apiculatus Hortob.* CLHE-H2. Agenţia de Stat pentru Proprietatea Intelectuală a Republicii Moldova. Depozit nr. a 2015 0027 din 18.03.2015. Chişinău, 2015.

[30]. Вассер С.П., Кондратьева Н.В., Масюк Н.П. и др. Водоросли: Справочник. Киев, «Наукова думка», 1989, 608 с.

[31]. Кожухарь И.Ф., Борш З.Т. Влияние карбонатной системы среды на культивирование водорослей при применении гидрокарбоната, как источника углеводного питания. Биологические ресурсы водоемов Молдавии. Кишинев, 1971, вып. 8, с.8-4

[32]. Кузин В.С., Овчаренко Э.В., Алфимцев Н.А., Алфимцева Г.М. Способ стимуляции продуктивности и плодовитости пчел. Патент изобретения RU 2 199 210 C2 2002.09.20, Калуга, 2003.

[33]. Панин А.Н., Мелник Н.И., Малик Е.В., Вершинина И.Ю. Биопрепарат для повышения продуктивности пчел. Патент изобретения RU 2 166 322 C2, Москва, 2001.

[34]. Плохинский Н.А. *Руководство по биометрии для зоотехников*. Изд. «Колос», Москва, 1989, 256 с.