

РЕЗИОМЕТА НА НАУЧНИТЕ ТРУДОВЕ

на гл. ас. д-р Свилен Богданов Лазаров

Представени за участие в конкурс за заемане

на академичната длъжност «Доцент» по научна специалност Специални отрасли (Пчели), професионално направление 6.3. Животновъдство,

област на висше образование 6. Аграрни науки и ветеринарна медицина,

обявен в ДВ бр. 56 от 19.07.2022г.

I. Резюме на Хабилитационен труд – монография.

Социално поведение на медоносната пчела (*Apis mellifera* L.)

Свилен Лазаров

1. Въведение

Медоносните пчели (*Apis mellifera*) са древни обитатели на нашата планета. Еволюционното им развитие ги е довело до обществен начин на живот. Те са социални насекоми и имат важна роля в опрашването на растенията, като от тях се добиват продукти с висока хранителна и стойност – мед, прашец, пчелно млечице, прополис и восък.

В таксономично отношение медоносните пчели принадлежат към разред Hymenoptera включващ оси, пчели и мравки. Към момента от този разред има около 150 000 живеещи вида и над 2 000 са изчезнали.

Пчелите са монофилетична линия в рамките на надсемейство Apoidea. Известни са над 16 000 вида пчели в седем признати биологични семейства. Те се срещат на всеки континент и във всяко местообитание на Планетата, което съдържа цъфтящи растения, опрашващи се от насекоми, с изключение на Антарктида.

Медоносните пчели принадлежат към семейство Apidae, което включва, както единично живеещи пчели, така и пчели, оформящи пчелни семейства. Значително голяма част от видовете живеят в диво състояние. Към групата на пчелите, образуващи семейства, спадат медоносните пчели от рода *Apis*, земните пчели (*Bombus* spp.) и пчелите без жило (род *Melipona* и род *Trigona*). Видовете от род *Apis* са групирани в три линии: пчели, гнездящи в кухини (хралупи) - *Apis mellifera*, *Apis cerana* и *Apis koschevnikovi*; малки пчели - *Apis florea* и *Apis andreniformis*; гигантски пчели - *Apis dorsata*. Настоящата класификация на медоносните пчели разграничава четири различни раси на *A. cerana*; а именно *Apis cerana cerana*, *Apis cerana himalaya*, *Apis cerana indica* и *Apis cerana japonica*.

Медоносните пчели *A. mellifera* L. са разпространени в цяла Европа, Африка. Към момента в света са признати 31 подвида на *A. mellifera*. Те обитават обширни територии, характеризиращи се с разнообразие от климат и растителност. Чрез естествен отбор в продължение на хиляди години са се обособили добре оформени популации на *A. mellifera*, които са идентифицирани, като подвидове, различни на морфологична,

етологична и молекулярна основа. На базата на популациите, които се характеризират със сходни биологични и стопански качества са създадени ценни породи медоносни пчели.

В световното пчеларство са признати и са се наложили 4 породи, които се отличават с ценни стопански качества и физиологично-поведенчески особености: Италианска (*Apis mellifera ligustica*), Сива планинска кавказка (*Apis mellifera caucasica*), Краинска (*Apis mellifera carnica*) и Европейска (*Apis mellifera mellifera*). Медоносната пчела, населяваща територията на България в таксономично отношение е най-близка до Краинската пчела (*Apis mellifera carnica*).

Медоносната пчела (*Apis mellifera* L.) е напълно социално насекомо и оформя пчелни семейства. Всяко семейство представлява добре организирана социална група със строго разделение на труда по отношение на снасянето на яйца, изхранване на ларвите, изграждане на восъчни пити, охрана на гнездото, събиране на храна и нейното съхранение. Между отделните членове на пчелното семейство съществуват добре развити механизми на комуникация, чрез различни видове танци, феромони, както и чрез обмен на течности (нектар, пчелно млечице и др.) между възрастни индивиди и между възрастни и ларви.

Поведението на пчелите е обект на изследователската работа на много специалисти в областта на пчеларството. Медоносните пчели имат високо развита нервна система и характерен сложен обществен начин на живот. Социалното поведение при тях е свързано с оцеляването на вида и противодействие на вредните фактори на заобикалящата среда. То се характеризира с функционална специализация и склонност на индивидите да се придържат заедно, като следват определена последователност и не допускат включване в състава им на чужди индивиди от техния собствен вид. Цялото поведение на пчелите се основава на рефлекторно-инстинктивна дейност, характеризираща се с несъзнателни действия.

II. Резюмета на статии, публикувани в научни издания, реферирани и индексирани в световноизвестни бази данни с научна информация

Резюме на статия Г.7.1.

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**Приложение на програма AutoCAD за измерване
хитинените части на тялото на пчели работнички
(*Apis mellifera* L.)**

**Application of AutoCAD Program To Measure Chitin Body Parts of
Worker Bees (*Apis mellifera* L.)**

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Abstract

Certain morphological characteristics of worker bees from 24 colonies are determined. 23 chitin body parts by two methods were measured: Standard method (Alpatov, 1948) – using a binocular loupe MBS-1 with a graticule at a magnification of 16 – 32 times, depending on the particular attribute; modern method using the program AutoCAD. To establish the accuracy of the methods was performed control measurement sections of graph paper and using the Calibration Block 1 and 10 mm (Certificate of Calibration N 1409914, Mitutoyo Corporation Miyazaki Plant, Japan). The results of the control measurement sections of graph paper showed that when working with binocular loupe identify errors (0.01/0.02 – 0.05 mm lens at 2 and 4 and 0.07 – 0.13 mm for lens 7), but using the program AutoCAD dimensions of chitin body parts of bees were obtained in millimeters and are undeviating. Results give grounds to state the following conclusions:

- Results of measurement with a binocular loupe MBS-1 (method of Alpatov, 1948) are obtained in increments, then multiplied by a factor depending on the increase to be recalculated in millimeters. Some chitin parts (length of the front wing, length of the proboscis) are measured in two parts. All these activities increase the potential for errors.
- AutoCAD program requires scanner with high resolution and computer. The amounts of chitin parts are automatically received in millimeters. Measurements are carried out quickly. Objects of measurement are scanned and can be stored for a long time.
- AutoCAD program can be successfully applied to determine the morphological characteristics of the body of the worker bees.

Genetic Comparison between Local *Apis mellifera macedonica* Ruttner, 1988 Selectively Reared for Production of Bee Queens and Swarms in Bulgaria and Honey Bee Colonies with Indicative Hygienic Behaviour

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Abstract: The genetic polymorphism of malate dehydrogenase (MDH-1) and esterase (EST-3) loci in selectively reared for production of bee queens and bee swarms in Bulgaria local honey bee *Apis mellifera macedonica* was studied in comparison with a group of honey bee colonies with indicative hygienic behaviour. Totally 851 worker bees collected from the selection bee rearing bases and 414 worker bee individuals from colonies with manifested hygienic behaviour were used for this comparative study. Both of the studied loci were found to be polymorphic all of the studied honey bee groups. Polymorphism with two and three alleles was found for MDH-1 locus and with four and five alleles – for EST-3 locus for compared hygienic colonies and those under selective control. Levels of polymorphism between 50% and 100% were calculated for the studied honey bee groups. The observed and expected heterozygosities (H_o and H_e), mean F_{ST} and Nm values were calculated and compared. The observed similarities and slight differences among honey bee groups under selective control and this with indicative hygienic behaviour were discussed.

Alloenzyme genetic characterization of *Apis mellifera* (Hymenoptera: Apidae) colonies from Bulgaria with different hygienic behaviour

Aloenzimna genetična karakteristika na pčelni semeystva *Apis mellifera* (Hymenoptera: Apidae) ot Bulgaria s različno higenno povedenie

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ABSTRACT

The genetic variability in 25 honey bee colonies from different regions of Bulgaria with different hygienic behaviour (highly hygienic, hygienic and non-hygienic) has been studied. Alloenzyme analysis of two systems (MDH-1 and Est-3) corresponding to 2 loci was used in order to characterize the colony polymorphism. Totally 1,150 worker bees were included in this investigation. MDH-1 locus was found to be polymorphic in all of the studied colonies, having two alleles – MDH-1⁴⁵ and MDH-1¹⁰⁰. The Est-3 locus was fixed in ten of the investigated colonies. Polymorphism with total presence of four alleles of this locus (Est-3⁴⁰, Est-3⁴⁴, Est-3¹⁰⁰ and Est-3¹¹⁴) was found in the other studied colonies. The calculated polymorphism was 50% in the non-hygienic and 100% in the highly hygienic and hygienic colonies. The observed and expected heterozygosities (H_o and H_e) ranged from 0.296 to 0.354 and from 0.28 to 0.332 in non-hygienic and highly hygienic groups, respectively. The calculated mean observed and expected heterozygosities were 0.32 and 0.307, respectively. The calculated F_{st} and N_m levels demonstrated lower differentiation between highly hygienic and hygienic colonies and higher differentiation between highly hygienic and non-hygienic colonies. Dissimilarities between levels of polymorphism, heterozygosity, F_{st} , N_m and allele frequencies in the studied groups of colonies with different hygienic behaviour were found and discussed. The results of the present study provide new information concerning relations between hygienic behaviour and alloenzyme characteristics which could be used for future selection with honey bees in Bulgaria.

Application of stimulating products in autumn feeding and wintering of the bee colonies (*Apis mellifera* L.)

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Abstract

Shumkova, R., Zhelyazkova, I. & S. Lazarov (2019). Application of stimulating products in autumn feeding and wintering of the bee colonies (*Apis mellifera* L.). *Bulg. J. Agric. Sci.*, 25 (Suppl. 3), 68–73

The effect of stimulating products on some parameters which characterize the wintering of the bees such as degree of the worker honeybees fat body, amount of dead bees and quantity of food consumption in the winter was investigated. For stimulating feeding of the bee colonies the products "Apimix", "Apipasta", "Probee-41" and "Baikal EM1" were used. The experiment was carried out in 2018 during the autumn feeding of the bee colonies at Research Center of Stockbreeding and Agriculture, town of Smolyan. Samples of worker bees were taken to determine the condition of the fat body at the end of the experiment before preparing of the bees for winter period. The degree of fat body development was determined by 5-point scale proposed by Maurizio (1954). For the characterization of the parameters of the wintering, two reviews of the bee colonies were carried out (during wintering in October 2018 and in March 2019). Strength of the bee colonies (amount of bees in the beehive) and amount of honey supplies (the capped honey in the honeycombs) were reported. It was found that the autumn feeding of the bee colonies with the addition of the microbiological product "Baikal EM1", the plant product "Probee 41" and the product "Apipasta" before the wintering has a positive effect on the development of the worker bees fat body. In the bee colonies which received with their food before wintering stimulating products "Apimix", "Apipasta", "Probee 41" and "Baikal EM1", the honey consumption for 1 kg of bees in the winter was reduced. The highest percentage of dead bees (winter loss) in the control groups of bee colonies C₁ and C₂ was established ($P \leq 0.05 - P \leq 0.01$). Winter loss under 20% was found in the colonies which received "Probee 41" and "Baikal EM1" during the autumn feeding.

Statistical Models for Assessing the Influence of Hygienic Behaviour of Worker Bees on the Level of Lysozyme and Total Protein Content in Their Haemolymph

Research Article

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ABSTRACT

The hygienic behaviour of 26 bee colonies of apiaries in different regions of Bulgaria was tested. The degree of expression of hygienic behaviour was evaluated by the pin-killing method. A test field of square 5 x 5 cm was stuck onto a section of a honeycomb with a sealed worker brood. The bee colonies are divided into two groups: hygienic (cleaned over 95% of cells in the testing area on the 48th hour) and nonhygienic (cleaned less than 95% of the cells in the testing area on the 48th hour). Haemolymph has been taken from bees from each bee colony at 48th hour and lysozyme levels and total protein content has been defined. Multivariate analysis (ANOVA) was used to determine significant differences between hygienic and nonhygienic colonies. The results obtained show significant differences between both groups ($P < 0.05$) according to percent cleaned cells after killing the brood at 3rd, 24th and 48th hours and insignificant differences between hygienic and nonhygienic colonies ($P < 0.05$) for both parameters-lysozyme and total protein. Bivariate correlation was applied to investigate the impact of hygienic behaviour on lysozyme levels and total protein content. Low negative correlation ($r = -0.33$) between total protein and hygienic behaviour was established which means that an increase of the activities related to the cleansing of honeycomb cells leads to a decrease in the total protein content. Low correlation ($r = 0.35$) between hygienic behaviour and lysozyme levels was found i.e. higher levels of lysozyme are reported with more pronounced hygiene behaviour. The calculated regression model for lysozyme ($P = 0.24826$) is statistically insignificant but the regression model for total protein is statistically significant ($P = 0.003153$) and can be used to predict the relation between total protein content in the haemolymph and hygienic behaviour of bee colonies.

Monitoring of temperature and humidity in hives made of different material by an electronic recording system during the autumn and winter period of the development of bee colonies

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Citation: Zhelyazkova, I., Lazarov, S., Germanov, D., & Mutafov, I. (2021). Monitoring of temperature and humidity in hives made of different material by an electronic recording system during the autumn and winter period of the development of bee colonies. *Zhivotnovadni Nauki*, 58(6), 47-59 (Bg).

Abstract

The changes of temperature and humidity in hives made of different material (wood, polystyrene, ceramics) during the autumn-winter period (2020–2021) from the development of bee colonies are tracked. Bee colonies settled in 10-frame hives Dadan-Blatt system with lattice and solid bottoms are used. To control the temperature and humidity in the beehives, an electronic recording system is used with sensors installed in each hive and one sensor for monitoring the outside temperature and humidity. During the studied autumn-winter period the temperature in the hives (average for the period and by months) is higher and the humidity lower compared to the values of the outside temperature and humidity, regardless of the material of the hive and the type of bottom (lattice or solid). The highest average temperature in the autumn period (September, October, November) is found in the ceramic hive with a solid bottom (29.15 ± 6.51 °C), and the minimum value is reported in the polystyrene hive with a solid bottom (19.01 ± 8.47 °C). During the same period, the maximum value of the humidity indicator for the hives with a lattice bottom is observed in the ceramic hive ($50.74 \pm 8.92\%$) and the minimum in the polystyrene hive ($45.91 \pm 9.62\%$). For beehives with a solid bottom, the highest average humidity is found in the polystyrene hive ($55.14 \pm 6.96\%$), and the lowest in the wooden hive ($44.69 \pm 5.00\%$).

During the winter period (December, January, February) the highest average temperature is found in wooden hives (10.15 ± 5.61 °C and 11.89 ± 5.16 °C), and the lowest in polystyrene hives (7.44 ± 5.12 °C and 7.61 ± 4.23 °C), regardless of the type of bottom. The largest difference from the outside temperature (5.11 °C on average) is observed in the wooden hives – 5.04 °C with a lattice bottom and 6.78 °C with a solid bottom, respectively. The lowest average value of the humidity indicator is reported in the wooden hives, regardless of the type of the bottom ($69.70 \pm 14.16\%$ and $55.45 \pm 10.04\%$), and the highest in the polystyrene hive with a lattice bottom and the ceramic hive with a solid bottom (in 70–75%). The difference between the average values of the humidity indicator in the hives compared to the external humidity (79.24%) in the hives with lattice bottoms is less than 10%. In the hives with solid bottoms the differences vary in the range of 6–24% with the highest being in the wooden hives.

Резюме на статия Г.7.7.



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Food consumption and winter mortality in bee colonies wintering in hives made from different materials with lattice and solid bottom

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(Manuscript received 10 June 2021; accepted for publication 2 August 2021)

Abstract. The objective of the present study is to examine the changes in some indicators characterizing the winter hardiness of bee colonies settled in hives made of different material (polystyrene, wood, ceramics) and with different type of bottom (lattice or solid). Some parameters which characterize the wintering of bee colonies (amount of dead bees and quantity of food consumption in winter) have been investigated. The bee colonies were housed in 10-frame Dadant Blatt hives with a lattice and solid bottom situated at the Training Apiary of the Faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria. Two inspections of the bee colonies were carried out (during wintering in November 2020 and at the beginning of the active season in March 2021). Amount of bees in the bee hive (strength) and amount of capped honey in the honeycombs were reported. In bee colonies wintering in hives with a lattice bottom, the consumption of food per 1 kg of bees was 1.639 kg (50%) higher than in hives with a solid bottom. Winter mortality of bees in hives with a lattice bottom was higher compared to this indicator in the hives with a solid bottom, $16.19 \pm 10.72\%$ and $12.59 \pm 3.57\%$, respectively, which can be considered excellent wintering below 15% and good wintering in the range of 15.0-19.99%.

Резюме на статия Г.7.8.

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Physicochemical characteristics of Bulgarian bee honey: Part 1

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Abstract

Lazarov, S., Veleva, P. & Zhelyazkova, I. (2022). Physicochemical characteristics of Bulgarian bee honey: Part 1. *Bulg. J. Agric. Sci.*, 28 (2), 349–354.

The present study investigates 59 representative samples of monofloral honey (Acacia 12 pcs., Coriander 12 pcs., Limetree 12 pcs., Rapeseed 11 pcs., Sunflower 12 pcs.) and 30 samples of multifloral honey, crop 2019, from various apiaries on the territory of the Republic of Bulgaria. The changes in the following physicochemical parameters (Active acidity (pH); Electrical conductivity, $\mu\text{S}/\text{cm}$; Water content, %; Refractive index; Fructose + Glucose (F+G) content, %) have been studied. The obtained average values of the above parameters conform to the requirements of Council Directive 2001/110/EC relating to honey and Regulation on requirements to bee honey intended for human consumption. Statistically significant differences have been observed between the physicochemical parameters of monofloral and multifloral honey. The values of the coefficients of determination (R^2) show that 77.8% of the variations in the variable for the parameter (pH) and from 22.4 – 36.5% in the other studied parameters are due to the effect of the type of honey.

Determining the amount of capped honey in honeycombs with AutoCAD program

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Citation: Lazarov, S., & Dineva, G. (2022). Determining the amount of capped honey in honeycombs with AutoCAD program. *Zhivotnovadni Nauki*, 59(1), 23-31 (Bg).

Abstract

The study was conducted with bee colonies from the local honey bee (*Apis mellifera* L.) at the Training and Experimental Base of the Beekeeping Section at the Agriculture Faculty of the Thrakia University, Bulgaria. The surface of capped honey was determined for 58 honeycombs. An established method in beekeeping with a measuring frame (squares 5/5 cm) was used and a comparative analysis of the data obtained when measuring the specified indicator with AutoCAD program was performed. A method has been established for accurate determination of the surface of capped honey in honeycombs using the AutoCAD program. The difference between the average value of the capped honey surface measured by the approved method with a measuring frame and using the program AutoCAD is 5.73%.



NUTRITIONAL EVALUATION OF YOGHURT PREPARED BY LACTOBACILLI ISOLATED FROM *APIS MELLIFERA* L. GUTS AND ALPINE ANTHILL

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Abstract

The use of different lactic acid bacteria with desired technological and probiotic characteristics requires their isolation from different sources. The aim of this study was to determine the influence of newly isolated lactobacilli from promising natural sources (bees and ants) on the quality of buffalo yogurt.

A total amount of 7 strains, isolated from bee guts (6) and alpine anthill (1), were tested as a starter culture for yoghurt preparation. Yoghurt was produced with the addition of 10% starter culture from the isolated strains. The titratable acidity (°T), coagulation time (min), macro- and microelement composition (by atomic absorption spectrometry), fat- and amino-acid composition (by gas chromatography), and the organoleptic properties of the yoghurt were studied.

The titratable acidity of the experimental yoghurt - 110 °T for *Lactobacillus casei* B4 (isolated from bee gut) and 116 °T for *L. casei* A1 (isolated from alpine anthill) was lower than the control value (139 °T). The coagulation time was the shortest in the control (168 minutes), followed by yoghurt with strains A1 (198 minutes) and B4 (240 minutes). The content of P, Zn and Cu was higher in the milk produced by the newly isolated strains A1 and B4. The atherogenic index ranged from 2.72 for raw milk to 2.47 for yoghurt produced by B4, 2.28 for yoghurt with A1, and 2.27 for control value. The same trend is observed in the thrombogenic index - from 1.74 for raw milk it decreases to 1.51 for B4, 1.42 for control and 1.04 for A1. The three types of yoghurt were characterized by a firm, tight coagulum with a granular structure and a pleasant lactic acid aroma.

In conclusion, from the 6 tested strains of *L. casei* and 1 of *Lactobacillus rhamnosus*, only A1 and B4 are suitable for production of high quality yoghurt, which in terms of beneficial effects on consumers' health should be not inferior to conventional yoghurt.

Key words: Yoghurt quality, Lactobacilli, *Apis mellifera* L., Alpine anthill.

1. Introduction

Lactic acid bacteria (LAB) are widespread in nature. They inhabit ecological niches rich in essential nutrients and energy, including plants and plant materials, soil, water, the intestinal tract of animals and humans, spoiled food [1 - 3]. Their distribution in nature is due to their diverse morphological, biochemical, physiological and taxonomic features, which are the basis for their adaptability [4].

The founder of the probiotics concept is considered to be the Russian scientist Ilya Mechnikov [5], who believed that the longevity of Bulgarian peasants is due to the consumption of yogurt, which contains LAB, whose growth in the intestine displaces pathogens. He believed that certain bacteria have a positive effect on the body health. The word probiotic (from Latin - pro and in Greek - bios literally means „for life“) was introduced by the German scientist Kollath [6] to denote „active substances that are essential for the healthy development of life.“ In 1989, Fuller described probiotics as „a living microbial, nutritional supplement that has a beneficial effect on the animal that takes it

General linear models based on physicochemical parameters of monofloral and multifloral bee honey: Part 2

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Abstract

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The objective of the present study is to establish regression models defining the relations between physicochemical parameters of bee honey and the type of honey. 89 samples of bee honey (acacia honey, coriander honey, lime tree honey, rapeseed honey, sunflower honey, and multifloral honey) collected from different regions of Bulgaria were used in the study. The following parameters have been defined: water content (WC); content of fructose (F), glucose (G), electrical conductivity (EC), refractive index (RI), and pH. Moderate to strong negative correlation between WC and RI has been found for all studied types of bee honey. In the samples from lime tree, coriander, and multifloral honey, positive but different in strength correlation has been observed – from weak to moderate correlation between pH and EC. The analysis of the samples with acacia honey showed differences in strength and direction of: lime tree and multifloral honey in terms of the correlations between pH and total F + G content; of lime tree, rapeseed, and sunflower honey in the correlations between F+G and RI and between WC and F + G. General Linear Models (1-5) have been compiled based on the connections between the considered physicochemical parameters and the different types of honey, which would allow subsequent estimation of the studied parameters.

Keywords: honey, physicochemical parameters, correlation analysis, regression analysis



AN INNOVATIVE METHOD FOR DETERMINING THE AREA OF THE CAPPED BROOD IN BEE COLONIES

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Abstract

The development of bee colonies is directly related to the number of worker bees and their supply of honey and pollen. This in turn affects the amount of brood (breeding). In modern beekeeping, this indicator is constantly monitored by beekeepers. There are well-established methods for its determination by using a measuring frame, a planimeter, roughly, by photographing the beehives and enumeration the cells with a brood, etc. The aim of this study is to develop and use a fast and accurate way to determine the area of the sealed worker brood in bee colonies using the program AutoCAD.

The experiments were performed with bee colonies from the local honey bee (*Apis mellifera* L.) at the training apiary of the beekeeping section at the Faculty of Agriculture of the Trakia University, Bulgaria. The area of the sealed worker brood on 46 bee combs was determined. The well-established methods (eye assessment method and using a measuring frame) in beekeeping are used as a comparative analysis of the obtained data is performed by measuring the specified indicator with the programs AutoCAD and Paint.

A fast and accurate method for measuring the area of the brood using the AutoCAD program has been established. Measurement accuracy of 99.35% was reported. The obtained average values compared to the classical method of measuring the area of the brood with a measuring frame do not differ significantly. The differences measured by these methods compared to the classical one are about 5%.

Based on the results obtained and the comparative analysis of the used methods it is recommended to use the AutoCAD program as an alternative and accurate

method for determining the area of the sealed brood in bee colonies.

Key words: Honey bees, Capped brood, AutoCAD.

1. Introduction

The development of bee colonies is directly related to the number of worker bees and their supply of honey and pollen [1]. This in turn affects the amount of brood (breeding). In modern beekeeping this indicator is constantly monitored by beekeeping specialists [1]. There are well-established methods to support for researchers its determination by using a measuring frame, a planimeter [2], roughly, by photographing [3, 4, 5, 6, and 7] the beehives and enumeration the cells with a brood, etc. The classical methods used to determine the amount of capped brood in the combs are slow and therefore a large number of samples cannot be processed [1]. These methods require a large team of beekeepers and technical staff and the probability of errors is higher [9, 10]. They have a number of inconveniences (time consuming, subjective mistakes are made) that can be avoided by computerizing the analysis. The computer method allows to reduce the time spent in the apiary. Automated tracking also improves data reliability. A number of authors report new and modern methods for monitoring the indicators characterizing the development of bee colonies [5, 8, 11, and 12]. The National Institute for Agronomic Research (INRA) in Avignon, France, cites publications using the ColEval method to determine the strength of bee colonies [13, 14, 15, 16, 17, 18, and 19]. Accurate and objective data on the strength of bee colonies can also be obtained by Uebefelder method [20]. The accuracy in determining the capped brood with this



REGRESSION MODELS BASED ON PHYSICO-CHEMICAL PARAMETERS OF BEE HONEY

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Abstract

Constantly increasing demand and consumption of honey establish it as a valuable and indispensable food product. This determines the need for high knowledge of the sources and conditions of extraction, proper storage, and knowledge of possible changes in its composition. In this regard, it is necessary to deepen and expand the physico-chemical analyzes of honey in order to preserve its valuable qualities and prevent its adulteration. The present study aims to determine some physicochemical parameters in Bulgarian bee honey and to compile and compare regression models defining the relation between physicochemical parameters and the types of honey.

The study is based on an experiment conducted during the 2019 beekeeping season. A total of 89 bee honey samples of different botanical origins (acacia, coriander, limetree, rapeseed, sunflower, and multifloral honey) were collected from apiaries located on the territory of the Republic of Bulgaria. To determine the botanical origin pollen analysis was performed at the Central Scientific Research laboratory at Trakia University, Bulgaria. The following physico-chemical parameters were studied: glucose + fructose (%) via liquid chromatography, and the detector used is differential refractometer; active acidity (pH) by pH-meter; electrical conductivity ($\mu\text{S}/\text{cm}$) by Conductometer with an electrical conductivity cell; refraction index and water content (%) by Abbe refractometer. Multivariate data analysis was applied to develop predictive regression models defining the connection between physico-chemical parameters and the type of honey. Four types of models were compared (linear, logarithmic, compound, and power) at $p < 0.05$.

It was found that for the parameters pH and electrical conductivity the most suitable is the compound model. The determination coefficients (R^2) showed that 4.7% of the variation in the data of the pH parameter and 14.5% of the electrical conductivity are accounted for by the type of honey. For fructose and glucose content, respectively, 15.6% of the variations depend on the type of honey. They are best described by the linear model. No statistically significant models have been found for the parameters water content and refractive index.

Studies can continue in the direction of searching for suitable models to describe the relation between these two parameters and the type of honey.

Key words: Honey, Physico-chemical parameters, Multivariate data analysis.

1. Introduction

„Honey is the natural sweet substance produced by *Apis mellifera* bees from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature.“ (2001/110/EC, 2001, [1]). Honey is a thick sugar solution. The main saccharides found in it are glucose and fructose, but there are other di- and tri-saccharides as well (Siddiqui *et al.*, [2]). Honey contains over 22 saccharides with fructose and glucose being in the greatest amount. The total amount of glucose + fructose reaches 75 - 80 % (Bogdanov *et al.*, [3]). The ratio between them is



Agriculture and Environment

Characteristics of some chitin body parts in worker bees (*Apis mellifera* L.) from hygienic and non-hygienic bee colonies

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Abstract. In the conducted study the level of manifestation of hygienic behavior of 25 bee colonies was tested from the honey bee (*Apis mellifera* L.) in Bulgaria. During the testing the method used for killing the brood was with an entomological pin. Based on the results two types of colonies were determined – hygienic (where 48 h after killing the brood the percentage of cleaned cells with dead brood was over 95%) and non-hygienic (where 48 h after killing the brood the percentage of cleaned cells with dead brood was lower than 95%). From each bee colony young non-flying worker bees were taken and the morphometric measurements of their chitin body parts were made. In the study 18 morphological traits were tested and 2 indexes were calculated. High degree of reliability ($p \leq 0.001$) between hygienic and non-hygienic bee colonies was determined for 2 morphological traits - Total length of fore wing and Length of proboscis. For the traits Length of tibia and Width of 1st tarsus of fore leg and Width and Surface of the left wax mirror of IIIrd sternit medium degree of reliability ($p \leq 0.01$) was determined. In 3 other traits - Length of femur of fore leg, Length of IIIrd sternit and Length of IInd part of the cubital cell of the fore wing low reliability ($p \leq 0.05$) was established. The results obtained from the present study indicate the existence of statistically significant differences between the morphometric characteristics of worker bees from hygienic and non-hygienic bee colonies. These traits are related to the productivity of worker bees.

Keywords: *Apis mellifera*, chitin body parts, hygienic bee colonies, non-hygienic bee colonies., morphometric measurements

III. Резюмета на статии и доклади, публикувани в нереферирани списания с научно рецензиране или публикувани в редактирани колективни толове.

Резюме на статия Г.8.1.

STUDY THE CONTENT OF LYSOZYME AND TOTAL PROTEIN IN THE HAEMOLYMPH OF HONEY BEES (*APIS MELLIFERA L.*) FROM BEE COLONIES WITH DIFFERENT HYGIENIC BEHAVIOUR

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Introduction

Honey bee (*Apis mellifera L.*) has a wide area of distribution and due to that the species shows exceptional ability to adapt to various environmental conditions (relief, climate, nectariferous plants, etc.).

The bee colony as a complete biological unit is able to apply different strategies of protection against adverse conditions and pests. In this regard, its immunity comprises a combination of social immunity of the colony and individual immunity typical of each specimen. Social immunity includes a number of instincts - swarming, self-replacement of the queen bee, leaving the infected nest, building the honeycombs, collection and transfer of propolis, formation of a winter ball, etc. This group of instincts includes the hygienic behaviour for cleaning the combs and the hive. The cleaning instinct as a behavioural response of bees along with orientation in the external environment and signalling, plays an important role in the formation of the productivity of the bee colony.

The immunity of the honeybee depends largely on the protein content in the haemolymph. In bees, as in all insects, mechanisms for building up an immune response are based only on natural protection, which includes several biochemical indicators (proteins - apidaecins, lysozyme and phagocytic activity, etc.). The concentration of these biochemical markers in the haemolymph of bees can be used as a criterion for determining the degree of hygienic behaviour of the bee colony.

A total of 30 different proteins have been found in the haemolymph of the honeybee (Lensky and Alumot, 1969). Their concentration is highly dependent of the ontogenetic development of the bee, the season, etc. (Lin and Dixon, 1965). One of the main factors of immunity in bees is the enzyme lysozyme (N-acetylmyramylhydrolase). According to a number of authors (Mohrig and Messner, 1968; Zumann, 1988; Gotz and Trenczek, 1991) lysozyme, the total amount of proteins and other antibacterial substances in the haemolymph

EFFECT ON THE CHEMICAL COMPOSITION OF THE BODY OF WORKER BEES (*APIS MELLIFERA* L.) FED WITH STIMULATING PRODUCTS

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The purpose of the work was to study the impact of stimulating products in the spring and autumn feeding of the bee families. More specifically, on the chemical composition of the worker bees body's of *Apis mellifera* L. The study was conducted in the beekeeping seasons (spring and autumn) in 2011 and 2012 at the experimental apiary of the Agricultural and Stockbreeding Experimental Station (ASES) – Smolyan, and at the apiary of beekeeping farm "Mitevi" in Smolyan. The bee families were fed with the products "Vita Feed Gold" (includes natural extract of beet and molasses in distilled water), "Apidas" (consisting of plant extracts), "Oligofosi" (contains amino acids) and "Anolyte-7" (electrochemically activated aqueous solution). Moisture (%), crude protein (%), crude fats (%) and ash contents (%) in the bee body are defined. During the spring the higher contents of crude protein and fat in the bee body of bee families received stimulating products "Vita Feed Gold" (in the apiary of ASES – Smolyan), "Apidas" and "Oligofosi" (apiary beekeeping farm "Mitevi") were found in comparison to the bee body of the bees from the control group. At the end of the beekeeping season the higher (relative to the control group bee families) are the protein and fat contents in the bee body which are received with the food "Apidas" and "Oligofosi". The use of activated water "Anolyte-7" as a sugar solvent leads to accumulation of protein and fat reserves in the bee body in the autumn feeding of the bee colonies. No significant differences in the ash contents of the bee body were found, irrespective of the stimulatory product and the season.

Application of stimulating products in autumn feeding and wintering of the bee colonies (*Apis mellifera* L.)

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Abstract

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The effect of stimulating products on some parameters which characterize the wintering of the bees such as degree of the worker honeybees fat body, amount of dead bees and quantity of food consumption in the winter was investigated. For stimulating feeding of the bee colonies the products "Apimix", "Apipasta", "Probee-41" and "Baikal EM1" were used. The experiment was carried out in 2018 during the autumn feeding of the bee colonies at Research Center of Stockbreeding and Agriculture, town of Smolyan. Samples of worker bees were taken to determine the condition of the fat body at the end of the experiment before preparing of the bees for winter period. The degree of fat body development was determined by 5-point scale proposed by Maurizio (1954). For the characterization of the parameters of the wintering, two reviews of the bee colonies were carried out (during wintering in October 2018 and in March 2019). Strength of the bee colonies (amount of bees in the beehive) and amount of honey supplies (the capped honey in the honeycombs) were reported. It was found that the autumn feeding of the bee colonies with the addition of the microbiological product "Baikal EM1", the plant product "Probee 41" and the product "Apipasta" before the wintering has a positive effect on the development of the worker bees fat body. In the bee colonies which received with their food before wintering stimulating products "Apimix", "Apipasta", "Probee 41" and "Baikal EM1", the honey consumption for 1 kg of bees in the winter was reduced. The highest percentage of dead bees (winter loss) in the control groups of bee colonies C₁ and C₂ was established ($P \leq 0.05 - P \leq 0.01$). Winter loss under 20% was found in the colonies which received "Probee 41" and "Baikal EM1" during the autumn feeding.

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Residual amounts of *Thiacloprid* in rapeseed (*Brassica napus*) and in bees

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Abstract

The aim of the present study is to establish the effect and residues of the neonicotinoid *Thiacloprid* in the treatment of rapeseed and its vector transmission in bees and honey. Samples of rapeseed (*Brassica napus*), bees and rapeseed honey were analyzed after treatment with the pesticide Proteus 110 U (active substance *Thiacloprid*). *Thiacloprid* concentrations in rapeseed, bee and honey samples were determined by high performance liquid chromatography with photodiode detection of HPLC-PDA by the method of Battu et al. (2008). It was found that the concentration of *Thiacloprid* in samples of roots, flowers and fruits of rapeseed is negligibly low - values close to the permissible maximum threshold (MRLs) of 0.5 mg/kg, in samples of bees is many times lower than that of the standard solution, but is read by the apparatus, and for honey samples very slight peaks below MRLs are observed and cannot be read by the apparatus. A high rate of degradation of the pesticide *Thiacloprid* was found - over 50% in the first 24 hours (56.1% for bee samples and 64.9% for plant samples).

УДК 638.1

**ИССЛЕДОВАНИЕ СОДЕРЖАНИЯ CHLORPYRIFOS
В РАПСЕ (*BRASSICA NAPUS*) И ПЧЕЛАХ
(*APIS MELLIFERA L.*)**

**STUDY OF CHLORPYRIFOS IN RAPESEEDS
(*BRASSICA NAPUS*) AND BEES (*APIS MELLIFERA L.*)**

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Ключевые слова: медоносная пчела, рапс (*Brassica napus*), инсектициды.

Аннотация. Исследовано влияние *Chlorpyrifos* (активное вещество широкоспекторного инсектицида Терагард Плюс) и его остаточного количества при третировании рапса (*Brassica napus*) и его векторная передача пчелам. Установлены остаточные количества инсектицида в цветках рапса, как и динамика его деактивации из организма пчел (более 9-ти дней). Полученные результаты показывают, что *Chlorpyrifos* является потенциальной угрозой как для летающих пчел, так и для пчелиной семьи в улье. Токсичность этого препарата для пчелиных семей требует безукоризненного соблюдения норм пользования, указанных в нормативной базе Республики Болгария.

14.09.2022 г.

гр. Стара Загора

С уважением

(гл. ас. д-р С. Лазаров)