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Report title	Performing a test of the effectiveness and safety of the ApiBrassin complementary feed in bees
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List of personnel participating in the study:

First name and last name	Tasks performed
Prof. Ph. D. Cezary J. Kowalski	study director
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Purpose of the study

The aim of the study was to establish the efficiency and safety of ApiBrassin supplementation in bees. ApiBrassin (a plant substance from buckwheat extract), containing in addition to known phytosterols - 24-epibrassinolide, which is one of the phytohormones obtained by bees in natural conditions. When used in accordance with the recommendations, it stimulates bee colonies, which results in increased egg production by queens. This increases the strength of the family, which increases the amount of honey produced. The content of 24-epibrassinolide in honey was determined by enzyme immunoassay. The study showed no significant changes in the mass fraction of 24-epibrassinolide after the use of ApiBrassin. The obtained results allow us to recommend without limitation the use of honey and bee products after using ApiBrassin.

2. Research methods

2.1. Tested samples

ApiBrassin mix for bees with three different serial numbers was used for the tests. The product was supplied by the manufacturer in packs of 2 glass ampoules, each with a capacity of 5 ml.

Composition of ApiBrassin (one 5 ml ampoule):

active ingredient: common buckwheat extract (*Fagopyrum esculentum* Moench) standardized to 0.2% 24-epibrassinolide; DER (Drug Extract Ratio) 200: 1

auxiliary substances: water 92%, ethyl alcohol 8%. Analytical composition: 24-epibrassinolide 0.2 mg / ml. Chemical name of the active substance: 24-epibrassinolide, (22R, 23R, 24R) -2 α , 3 α , 22,23-tetrahydroxy-B-homo-7-oxa-24-methyl-5 α -ergostane-6-one (natural 22R, 23R-isomer). Physical properties: colorless, transparent liquid with an alcohol odor, soluble in water and other solvents. Storage conditions: store in a dry and dark place. Period of validity: 3 years.

2.2. Determining the safety of Apibrassin in apiaries

Safety tests of the mixture for ApiBrassin bees were carried out in apiaries in the Zagańsk commune on 09.09-09.10.2020. The subject of the research were bees of the Carpathian breed. The observations were carried out for 6 control (I) and 6 experimental (II) colonies with an average of 6 alleys. The bee colonies, depending on the colony's strength, were fed sugar syrup in the amount of 18-20 kg during winter feeding.

In the experimental group of bee colonies (II), 1 ml of ApiBrassin mix for bees was added to the syrup in the amount of 1 ml per 1 liter of syrup. The number of brood and eggs released was chosen as an estimate of the feed safety index.

2.3. Safety determination of Apibrassin in isolation

Two frames with bees were placed in insulators at room temperature and relative humidity not greater than 65%. In order to determine the harmlessness, bees from one of the exposed frames (II) were administered sugar syrup in the amount of 300 ml containing 0.3 ml of ApiBrassin. The bees in the exposed control frame (I) were fed sugar syrup in the same amount without the ApiBrassin bee additive. The bees were observed for 6-8 days. At the end of the observation period, dead bees from both frames were counted.

2.4. Methods for determining the development of the bee family when using Apibrassin

Depending on the strength of the colony, during the autumn feeding, in September 2020 bee colonies were given sugar syrup in the amount of 18-20 kg. For the experimental group, the additive for bees, ApiBrassin, was added to the syrup in the amount of 1 ml per 1 liter of syrup. In order to increase the strength of bee colonies, in the spring of 2021, ApiBrassin was applied 3 times with an interval of 4-5 days in the amount of 1.5-1.6 liters per bee colony 6-7 lanes. Then, during the summer period, the same amount of the period of low productivity of the bees was administered 2 times with an interval of 6 days. Assessment of the development of bee colonies with the use of the ApiBrassin mixture for bees was carried out in apiaries in the Zagańsk commune in March-August 2021. Carpathian bees were the subject of the research. Observations were carried out for 6 control (I) and 6 experimental (II) bee colonies with an average of 6 streets. The development of bee colonies was assessed on the basis of the results of monthly counts, taking into account the following indicators: strength of bee colonies - by counting the number of full alleys occupied by bees and the number of brood produced. The number of brood was determined by means of a frame inside which a wire was stretched, creating 25 cm² squares with 100 cells of bees. Only the brood produced is taken into account. Based on these data, the average daily production of queen bee eggs was calculated. The bees' volatile activity was determined by counting the bees returning to the hive within 3 minutes, daily from 10.00 to 12.00 for 3 days during the low flow period. Honey yield was determined by the amount of honey selected from the bee colonies at the end of the honeyflow.

2.5. Determining the conditions for the use of apiculture products

The subject of the research were 4 bee colonies of the Carpathian line, of which 2 colonies (II) received ApiBrassin as an addition to the sugar syrup. The feeding of the bee colonies was carried out on June 30, 2021. During the research, bees were given 2 liters of ApiBrassin sugar syrup in the amount of 1 ml per 1 liter of sugar syrup. Control colonies (I) received only sugar syrup. Dead bees were collected 5-10 days after the start of feeding, and honey was collected into combs.

2.6. Methods for the determination of 24-epibrasinolide in beekeeping products

Mass fraction of 24-epibrasinolide in honey, honey foam, bee pollen, and in bee extract was determined by the enzyme immunoassay method using a kit for the determination of 24-epibrasinolide (Institute of Bioorganic Chemistry of the National Academy of Sciences, Belarus, Belarus). Bee extract was prepared in an alcoholic solution. The test results are presented as mean values from these analyzes.

3. Research results

3.1. Apibrassin safety rating

In the period of safety tests for ApiBrassin bees in apiaries in the Zagańsk commune in the period from September 21 to October 9, 2020, there was no mass death of bees and no decrease in the number of streets compared to the inspection. Moreover, an increase in the mean brood count in the colonies administered ApiBrassin with the sugar syrup was found. The queens in the colonies of the experimental group (II), despite the weather conditions and the corresponding season (autumn), began to lay eggs. This indicates stimulation of the uterine reproductive function under the influence of the bioactive ApiBrassin top dressing. The results of the tests performed are presented in Table 1.

Table 1. Comparison of the number of broods and the number of eggs in the control and experimental bee colonies.

	Number of broods	Number of eggs
Control colonies (I)	3,66±1,60	-
ApiBrassin (II) bee colonies	7,00±2,04	19,17±7,01

When determining the safety of ApiBrassin on the bee frames in the isolators, no significant differences were found between the number of dead bees in the bee control isolator (I) and the bees that received 300 ml sugar syrup (II) containing 0.3 ml ApiBrassin. The results of the control of the number of dead bees during the observation period are presented in Table 2.

Table 2. Comparison of the number of dead bees in frames from control and experimental colonies.

Registration days after landing in isolation	Number of dead bees, pcs.	
	Control frame (I)	Bee Frame with ApiBrassin (II)
3	146	89
6	367	177
8	544	343

3.2. The influence of Apibrassin on the development of the bee family

The results of the research show (Tab. 3) that after the application of spring supplementation stimulating the laying of control mothers (I), it remained at the level of 460-842 eggs per day in March and April 2021. In families that received ApiBrassin as part of spring supplementation (II), this number increased by 1.4-1.7 times and amounted to 779-1137 eggs per day. Throughout the observation period (March-July), higher production of mothers' eggs was recorded in the bee colonies of ApiBrassin (II) compared to the control (I). In mid-July, this number reached its maximum and amounted to 2008 eggs / day in the group of bee colonies (II) against 1443 eggs / day in the control group (I).

Table 3. Comparison of egg production in control and experimental colonies.

Registration dates	Production of queen bee eggs, eggs/day	
	Control colonies (I)	ApiBrassin (II) bee colonies
18.03.2021	460±30	779±46
16.04.2021	842±33	1137±49
17.05.2021	1474±79	1876±24
17.06.2021	1544±68	1927±32
15.07.2021	1443±52	2008±20

It should also be noted that feeding the colonies with ApiBrassin did not increase their aggressiveness. According to our observations, the number of emerging colonies in the experimental and control bee colonies was approximately the same.

The bees' aviation activity was assessed in the period of May 17-19, 2021, between 10.00 and 12.00 (Table 4). As a result of the 3-minute counting of bees returning to the hive, it was found that in the bee colonies that received ApiBrassin (II) the number was 137 ± 15 bees (from 98 to 189 bees within 3 minutes), and in the control group, the colonies (I) 68 ± 13 bees (from 25 to 98 bees). As a consequence, the families that received the ApiBrassin (II) preparation were characterized by significantly greater aviation activity, which can be explained by more intensive development compared to the control families, as well as by a better physiological condition.

Table 4. Summary of bees' aviation activity in control and experimental colonies.

Registration dates	Aviation activity, bees/3 minutes	
	Control colonies (I)	ApiBrassin (II) bee colonies
17-19.05.2021	68±13	137±15

3.3. The influence of apibrassin on honey production

The increase in the strength of bee colonies and the flying activity of worker bees had a significant impact on their honey yield. In the bee colonies that received ApiBrassin (II) as an addition to sugar syrups, in the season of 2021 an average of 24.9 ± 2.0 kg of honey was obtained, which is 49% more than in control (I) families - $16.7 \pm 1, 3$ kg.

4. Determining the conditions for the use of bee products after the application of Apibrassin

Data on the content of 24-epibrasinolide in dead bees as well as apiculture products after feeding with ApiBrassin are presented in Table 5. The typical indicator of 24-epibrasinolide content in plants was taken from milk thistle seed extract, where, according to the research data, the mass fraction of 24-epibrasinolide was $1,5 \cdot 10^{-6}\%$.

Table 5. Comparison of the % share of 24-epibrasinolide in bee products obtained from control and experimental colonies.

Name of the research subject	Mass fraction of 24-epibrasinolide, %	
	Control colonies (I)	ApiBrassin (II) bee colonies
Honey foam	$8,6 \cdot 10^{-6}$	$8,2 \cdot 10^{-6}$
Bee pollen	$5,2 \cdot 10^{-6}$	$5,9 \cdot 10^{-6}$
Honeycomb	$3,2 \cdot 10^{-6}$	$5,3 \cdot 10^{-6}$
Bee extract	$0,5 \cdot 10^{-6}$	$5,8 \cdot 10^{-6}$

Thus, the percentage of 24-epibrasinolide in honey and bee products in colonies after the application of ApiBrassin (II) remained at a very low level, comparable to its natural content in various plant products consumed by humans. The obtained data on the mass fraction of 24-epibrasinolide in the bee extract after the application of ApiBrassin (II) turned out to be slightly higher than in the control, but at about the same level as in the honey foam taken from bees that did not receive ApiBrassin (I) and within the error limits in this measurement method.

5. Conclusions

ApiBrassin complementary feed for bees is harmless for the bees. The use of ApiBrassin in accordance with the manufacturer's recommendations stimulates the development of bee colonies and effectively increases the honey yield of bees. Beekeeping products after applying ApiBrassin can be used without restrictions.