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# Hygienic behavior of bees as an element of sterility in the production of environmentally friendly products

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Abstract. Beekeeping in Russia has been widespread for a long time and almost everywhere, a traditional and significant branch of the country's agriculture. In the Russian Federation, as in most countries, beekeeping is mainly practiced by amateurs. In 2019, according to government statistics, there were about 4.0 million bee colonies in the country, with more than 90% of them in the private sector. One of the factors of high productivity of bee colonies is the hygienic activity of individuals. The behavior of the bees in maintaining cleanliness and hygiene in the hive is astonishing. Not only the honeycomb, but even the walls and floor of the hive are always kept neat, polished with propolis. Garbage falling to the bottom of the hive is immediately thrown out through the entrance. Moreover, large litter, for example, the corpse of a bee, is pulled out by the orderlies onto the boarding board, and, grabbing it with their legs, take off to carry it away from the hive. Worker bees sweep small debris out of the hive with their fan wings, like brooms, and even brush dust particles to the tap hole. Sterility in a honey bee nest can be compared to an operating room in a modern clinical hospital.

#### 1. Introduction

The instinct of neatness in honey bees is so strong that during several months of wintering they, unable to fly out, abstain from defecation and only during the first spring flyby do they clear themselves in the air.

In a bee family there are 70–80 and more thousand insects. Such an accumulation of individuals will not be able to exist without the support in the nest of the necessary order and cleanliness. In all this, the instinct of self-preservation is manifested. Thanks to such neatness, bees warn themselves against possible diseases, get rid of wax moths and tiny insects willingly eating their garbage and not at all safe and not harmless for bees [1,2,3].

The genetically determined hygienic behavior of middle-aged workers plays an important role in the life of the bee colony. It includes the identification of diseased individuals at the larval and pupal stages of development and the removal of all infected brood, which reduces the intensity of the pathology. Bee colonies consisting of young bees remove dead brood without regard to bringing nectar to the hive, in

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contrast, colonies consisting of bees over 4 weeks of age removed dead larvae only when nectar entered the hive. Other researchers also noted the influence of the age of bees on their hygienic behavior [1,2,4,5].

In this regard, the purpose of our work is to study the level of hygienic behavior of bees using the method proposed by Milne – removal of frozen brood. For this, the following tasks were solved: to assess the strength of bee colonies in the spring; study methods for assessing the hygienic behavior of bee colonies; determine the intensity of removal of the killed brood of colonies; determine the honey productivity of bee colonies; determine the economic efficiency of research results.

The genetically determined hygienic behavior of middle-aged workers plays an important role in the life of the bee colony. It includes the identification of diseased individuals at the larval and pupal stages of development and the removal of all infected brood, which reduces the intensity of the pathology [1,5].

Hygienic behavior is one of the important qualities of the honey bee. It is the main defense against American foulbrood and fungal diseases, and against varroa mites. Testing for this sign was carried out by the method proposed by S.R. Milne. It involves freezing a portion of the sealed brood bee and counting the number of cells cleared by the bees in 24 hours. This can be done in two ways, one of them is freezing part of the brood in the refrigerator for 24 hours (FKB). Another is the liquid nitrogen killed brood test (LNKB). For freezing, use 3–10 day old pupae (only pupated to a light cinnamon color). We used the LNKB method. For the LNKB experiment, we made a 5x5 profile pipe and cut out a 7 cm high section from it, the brood freezing area was 100 cells [1,2,4].

Before starting the experiment, a section of sealed brood (pupae of suitable age) was taken and a 5x5 square tube was placed over it. We pressed the pipe so that, during freezing, liquid nitrogen did not get into neighboring cells. For liquid nitrogen, a 150 ml cup was used. About 1/4 of the volume of the cup was poured, when the liquid nitrogen evaporated, the residue was added to a total volume of 150 ml. We waited until the liquid nitrogen evaporated, the pipe thawed, then the pipe with the bee frame was removed. And they left the frozen printed brood in the families for 24 hours.

We put a mark on the top of the frame to make it easier to find it when counting the cells, and returned the frame to the colony. After 24 hours, the cells that were cleaned and remained unopened with pupae were counted. A bee colony is considered hygienic if it completely removes at least 90% of the frozen pupae within 24 hours.

The strength of a colony is determined by the number of working bees in it; two indicators are used to characterize it (depending on the required degree of accuracy): the live weight of bees (in kilograms) and the number of streets occupied by bees (a street is the space between two neighboring cells), streets in one street can reach up to 6000 pieces. In industrial conditions, the strength of a colony is determined by the number of frames covered by bees, not streets. This is one and the same, since on each bee frame there is half of one street of bees -3000 pieces, and on both sides of the bee frame there are bees, as in a whole street [1,5,7,8].

Thus, measurements of the strength of a colony – live weight in kilograms, the number of bees in pieces and the number of streets they occupy – can be translated into each other with minor errors. In the hive, the mass of bees depends on their age, young bees weigh 127–135 mg, and adult flight bees weigh 75–86 mg. The bee colony consists of adult and young bees, the average weight of a working bee in a bee colony is taken into account as 100 mg. So in 1 kg of the Carpathian bee breed, there are about 10 thousand individuals of all ages. It is generally accepted that on the Dadanov frame or between two frames (in the street) an average of 210–245 g of bees is placed. Consequently, a colony sitting on 10 streets has a mass of 2.1 to 2.45 kg and contains 21–24 thousand individuals.

To determine the live weight of bees in a family, they are shaken out from the honeycomb into a plywood box through a wide funnel (having previously determined its weight), and the remaining individuals are swept away with a brush. Beekeepers carry out work before the flight of bees – early in the morning. After determining the live weight of the bees, they are poured back into the bee hive. The mass difference between the empty box and the box with bees determines the live weight of the colony. In this method, there is a high probability of disturbing the bees, disrupting their normal work for the

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whole day, as well as in this method, high labor costs, so it will be used if accurate data is required for the purposes of scientific work.

The method proposed by V.V. Malkov, allows not to disturb the normal rhythm of bees, the essence of the method is to determine the amount of printed brood in the nest using a grid frame divided into squares. Each 5x5 cm square contains 100 bee brood cells. A grid frame was placed on both sides of the brood comb and the number of complete squares occupied by the printed brood was counted [1,8].

Bee brood is sealed for 12 days, since bees live about 36 days during the active season, then the count is carried out 3 times, every 12 days, that is, all bees that have left the family for 36 days are taken into account. The sum of the printed brood of 3 consecutive counts serves as an indicator of the number of bees in the colony on the 12th day after the last count. A comparative assessment of the determination of the number of bees in colonies by different methods showed that the sum of three counts of printed brood gives a fairly accurate idea of the strength of the colony. The calculated number of bees can be converted into their mass, based on the following ratio: the mass of 10 thousand bees is 1 kg.

#### 2. The results and discussion

The main goal of the spring audit is to find out the state of each colony after wintering and create conditions for intensive rearing of bees for honey harvest. During the spring revision, the strength of the families, the amount of brood and feed, the presence and quality of the queen are taken into account. The condition of families can be determined without completely removing the honeycomb from the nest, but only slightly lifting them above the hive and moving them from one wall to another. The strength of families can be determined by the number of streets occupied by bees. Families occupying 8–9 streets, average 6–7 and weak 4–5 streets or less are considered strong during this period.

The presence of a queen is determined by the presence of brood and the behavior of bees, that is, the absence of signs of behavior that characterize the state of queenlessness. By moving the combs apart with a chisel, the presence of laid eggs and a printed brood can be seen confirming the presence of a queen.

Raising or rearranging the honeycomb, determine by eye the amount of honey contained in each of them. If necessary, 1–2 empty frames are removed from the hive and combs with good-quality food are given instead.

In the bee farm in the spring, we observed the growth and development of families of the carpathian and krajinskaya breeds. In early spring, in our observations, we counted the number of streets covered by bees, and with the help of a grid frame, we determined the strength of the bee colony. In the studies, 4 full-fledged bee colonies were used, two of which are carpathian breed and two krajinskaya breed. Table 1 shows the comparative characteristics of the strength of families in the spring.

**Table 1.** The strength of families of the Carpathian and Krajina breeds in the spring.

		Breed			
Index		carpathian		krajin	skaya
		1	3	2	4
	26.03.2021				
Number of streets occupied by bees, pcs.		4	4	4	4
Number of squares of printed brood 5x5 cm in		58	56	60	61
a bee colony, pcs.					
	07.04.2021				
Number of streets occupied by bees, pcs.		5	5	5	5
Number of squares of printed brood 5x5 cm in		81	90	84	85
a bee colony, pcs.					
	19.04.2021				
Number of streets occupied by bees, pcs.		6	6	6	6
Number of squares of printed brood 5x5 cm in		120	108	112	116
a bee colony, pcs.					

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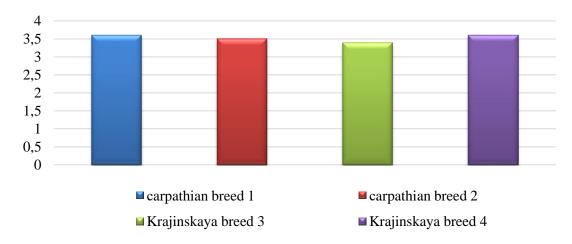
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	01.05.2021				
Number of streets occupied by bees, pcs.		8	8	8	8
Number of squares of printed brood 5x5 cm in		135	151	162	167
a bee colony, pcs.					

The number of printed brood in colonies did not differ significantly over the observation period from 03.26.2021 until 01.05.2021 in the experimental and control groups. This indicates a fairly uniform development of all observed families and the possibility of conducting comparative studies.

Using the method of calculating the brood to determine the strength of the colony, it can be noted that in the bee colony 1 for the period from 04.07.2021 until 01.05.2021 there were 336 5x5 squares (100 cells) of printed brood, therefore there were 33,600 cells in the family. Thus, by May 14, 2021. there were about 3.6 kg of bees of different ages in the family. In families 3, 2, 4 by 16.05.2021 there were 3.4 kg, 3.5 kg, and 3.6 kg, bees, respectively (the calculation was carried out in the same way).



**Figure 1.** Comparison of the strength of families of the carpathian and krajina breeds.

Figure 1 shows that families 1 and 4 have the same strength of 3.6 kg, while families 2 and 3 are inferior to them by 0.1 kg and 0.2 kg, respectively. Consequently, families of the carpathian breed have a strength of 7.1 kg, and families of the krajinskaya breed of 7.0 kg, which indicates an equal development of the observed bee colonies.

In studies on the behavior of bees, several methods are known for assessing their hygienic activity. Methodology for assessing the hygienic behavior of bee colonies according to Krivtsova L.S. consists in determining the intensity of removal by bees of pieces of paper placed at the bottom of the hive. It was proposed to determine the amount of paper removed by the change in its weight before and after placement in the hive.

The method for assessing the hygienic behavior of bee colonies according to Newton and Ostasiewski is that the colonies mark the area on the frame with the sealed brood, on which the pupae of the bees are killed, by piercing the cells with a needle, and then the intensity of the opening and cleaning of the cells with the killed brood by the bees is determined.

The method for assessing the hygienic behavior of bee colonies according to Milne differs from others in that it includes freezing a part of the sealed brood with liquid nitrogen and taking into account how many dead pupae the bee will remove within 24 hours, the more the honeycombs are cleaned of dead pupae, the higher the hygiene in a bee colony [2,8]. This method was applied in the farm on bee colonies of the carpathian breed and the krajinskaya breed. Table 2 shows the ability of different bee colonies of different breeds to fight dead brood.

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	Group				
Index	control		experienced		
	carpathian 1	carpathian 3	krajinskaya 2	krajinskaya 4	
	17.05.20	21–18.05.2021			
Bee brood killed, pcs.	100	100	100	100	
Cells opened by bees, pcs.	55	13	91	97	
Removed dead pupae, pcs.	50	10	90	97	
Cleansing speed of cells, pcs/hour	2.1	0.4	3.75	4.0	
	02.06.202	21-03.06.2021			
Bee brood killed, pcs.	100	100	100	100	
Cells opened by bees, pcs.	100	87	100	83	
Removed dead pupae, pcs.	78	87	100	85	
Cleansing speed of cells,	3.25	3.6	4.2	3.5	

**Table 2.** Ability of bees of different breeds to clean up killed bee brood, in 24 hours.

Table 2 shows that the family of the carpathian breed 1 for the period of the experiment from 05.17.2021 on 05.18.2021, in 24 hours opened 55/100 pcs. dead cells, removed 50/100 pcs. dead pupae with a purification rate of 2.1 cells per hour. For the period of the experiment from 02.06.2021. to 06.03.2021, in 24 hours opened 100/100 pcs. dead cells, removed 78/100 pcs. dead pupae with a cleaning rate of 3.25 cells per hour. Family of the carpathian breed 3, for the period of the experiment from 05.17.2021 on 05.18.2021, in 24 hours I opened 13 pieces dead cells and removed 10 pcs. out of 100 dead pupae with a cleaning rate of 0.4 cells per hour. For the period of the experiment from 06.02.2021. until 03.06.2021, 87 pieces were opened in 24 hours dead cells and removed 87 pcs. dead pupae with a cleaning rate of 3.6 cells per hour.

We also observe that the family of the krajinskaya breed 2, for the period of the experiment from 05.17.2021 until 05.18.2021, in 24 hours I opened 91 pieces dead cells, removed 90 pcs. dead pupae with a cleaning rate of 4.0 cells per hour. Experiment from 02.06.2021 on 03.06.2021 showed that, in 24 hours, 100 pieces were opened by bees dead cells, the family removed 100 pieces, that is, one hundred dead pupae out of a hundred, with a cleaning rate of 4.2 cells per hour. Family of the krajinskaya breed 4, for the period of the experiment from 05.17.2021 on 05.18.2021, in 24 hours I opened 97 pieces dead cells, removed 97 pcs. dead pupae with a purification rate of 3.75 cells per hour. For the period of the experiment from 06.02.2021 until 03.06.2021, opened 85 pieces in 24 hours dead cells, removed 83 pcs. dead pupae with a purification rate of 3.5 cells per hour.

**Table 3.** Hygienic activity of bee colonies of the Carpathian and Krajina breeds (05.18.2021).

Index		M±m		Cv,%	
	control	experienced	control	experienced	
Cells opened by bees, pcs.	34±5.9	94.0±6.9	24.0	10.3	
Removed dead pupae, pcs.	30±5.5	93.5±6.8	25.6	10.2	

Having studied the hygienic activity of the experimental and control groups on may 18.2021, it can be seen that in 24 hours the carpathian bee colonies opened 34±5.9 cells, removed the dead pupae 30±5.5, and the families of the krajinskaya breed coped with these indicators by 60% and 63.5% better, respectively.

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<b>Table 4.</b> Hygienic activit	y of bee colonies of the	Carpathian and Krajina	breeds (03.06.2021).
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Index	]	M±m		Cv,%		
	control	experienced	control	experienced		
Cells opened by bees, pcs.	93.5±6.9	91.5±6.8	10.4	10.5		
Removed dead pupae, pcs.	82.5±6.5	92.5±6.8	11.0	10.4		

The hygienic activity of the carpathian breed bee colonies on 06.03.2021 showed that in 24 hours  $93.5\pm6.9$  cells were opened, the dead pupae  $82.5\pm6.5$  were removed, and the bee colonies of the krajinskaya breed coped with this, respectively, by  $91.5\pm6.8$  and  $92,5\pm6.8$ . The deviation in both groups for these indicators is low, since it does not exceed 15%.

Figures 2 and 3 show a comparison of breeds in terms of hygienic activity for the period 17.05.2021 –18.05.2021 (experiment 1) and 02.06.2021–03.06.2021 (experiment 2) by such indicators as: the number of cells opened by bees and the number of removed pupae in 24 hours.

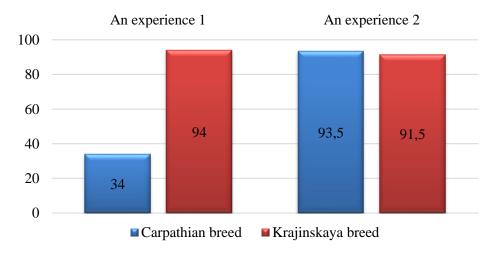
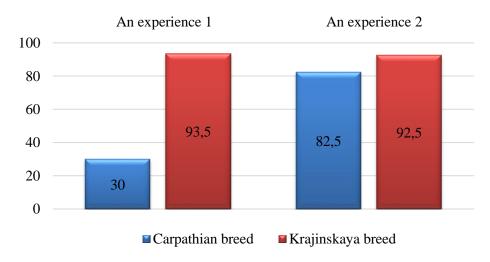


Figure 2. The number of cells opened by bees.

Thus, in 24 hours the krajinskaya breed of 05.17.2021-05.18.2021 opened 60% more bee cells than the carpathian, and 06.02.2021-03.06.2021, both breeds coped only 90%.



**Figure 3.** The number of dead pupae removed.

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With the removal of dead pupae on 05.17.2021–05.18.2021, the krajinskaya breed coped 63.5% better than the carpathian, and during the experiment period from 06.02.2021 to 06.03.2021, the krajinskaya breed performed this work 10% better.

Honey is a high quality dietary food product and a remedy for many diseases. Do not think that bees collect honey only for the needs of people. Honey is the main food supply for bees and their offspring (larvae, or brood), especially in the cold season. Therefore, when assessing the honey productivity of bee colonies, it is important to subdivide all honey stored by bees into fodder and commercial. It is possible to successfully develop beekeeping and obtain high quality marketable products only if there are good natural or cultivated honey plants [8,9].

During the month of May, the farm received marketable honey from the test families. Table 5 shows the amount of obtained commercial honey for the month of May 2021.

Index	Group				
	control experienced				
	carpathian 1	carpathian 3	krajinskaya 2	krajinskaya 4	
Nectar delivery, kg	24.0	22.0	30.0	28.0	
Received marketable honey, kg	12.0	11.0	17.0	15.0	

**Table 5.** Honey productivity of bee colonies for the month of May 2021.

Table 5 shows that during the period of honey collection in the month of may 2021, 32 kg of commercial honey was obtained from the bee colonies of the experimental group, and 23 kg from the control group. The delivery of nectar in the experimental group exceeded the delivery of nectar in the control group -1.3 times.

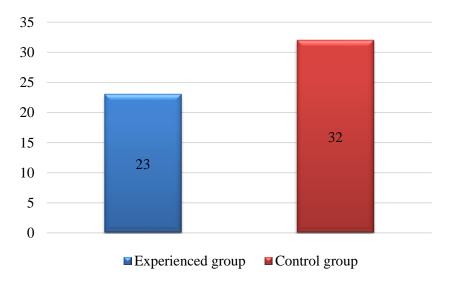


Figure 4. Produced commercial honey.

During the honey collection period, it can be seen from figure 4 that the control group brought 9 kg of commercial honey less than the experimental one, possibly due to the fact that the experimental group bees were more active and efficient during the honey collection period compared to the control group.

#### 3. Conclusion

Making a profit is the main goal of any production. Profit is one of the types of financial result, which is formed on the accounts of sales, accounting for other income, etc. In modern conditions of beekeeping

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development, substantiation of the economic feasibility of using bees of different breeds is of great importance [5,6,9].

In our studies, the obtained results of bee production, experimental bee colonies, show that with equal monetary costs (2132.0 rubles) for the control and experimental group, from the bee colonies of the carpathian breed, a net profit of 9368.0 rubles was obtained, and from the bee colonies of the krajinskaya breed 13868.0 rubles, which is 4500.0 rubles net profit is greater than that of the control group. At the cost of honey 500.0 rubles per kilogram. The level of profitability in the experimental group was above 85%, which indicates the high efficiency of using the families of the krajinskaya breed, which have the best hygienic properties.

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