THE USE OF SOME EXOMETABOLITES FROM MICROMYCETES FOR THE FORTIFICATION OF RESISTANCE INDICES IN BEE

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Abstract

Abstract. The goal of the proposed research was focused on the use of exometabolites of micromycetes to increase the physiological resistance of bee families after the winter period, as well as to stimulate their productive indices. From the 21 strains of micromycetes taken from the National Collection of Nonpathogenic Microorganisms of the Institute of Microbiology and Biotechnology, TUM, were selected 3 strains (Ps.sp.11, Ps.sp.19 and Ps.sp.62) which showed more productive indices of the development on culture media, as well as more pronounced bactericid properties. Exometabolites were prepared from the mentioned strains and administered to 3 experimental grups of bee familes in doses of 10, 25 and 50 ml per kg of wheat flour cakes. The productive indices of the bee families were examined over 12, 24 and 36 days after the administration of the biomass of exometabolites. As a result, it was established that the highest index - 47.1 squares of hatched brood, was registered at 24 days after the administration in the 1st experimental group of bees which was fed with a dose of 25ml/kg of wheat flour cakes. The difference between this group and the control group was 19.4 squares of hatched brood. At the same time, the honey collection per beech was 3.4 kg in the 1st experimental group of bees, representing 0.8 kg more compared to the control group and the prolificacy index was 34.5% higher compared to the control group.

Key words: exometabolites, micromycetes, bees, honey, culture media, prolificacy

Introduction

All bee species are extremely important to balancing different ecosystems. Due to bees, many plant species are pollinated in forests, agricultural plants, fruit trees and other various ecosystems; resulting in the production of fruits, vegetables and cereals, which serve as food for humans and animals. It is known that worldwide more than 300 species of cultivated plants are totally or partially dependent on pollination, and the production of 75% of the crops that provide products traded on the world market depends on pollination. In some agricultural crops (over 90), bees increase production by at least 30% (cotton, medicinal plants, agricultural crops, animal feed), and about 10% of entomophilous agricultural crops depend entirely on bee pollination [4,7].

The bees are among the most evolved social insects, which means that they jointly carry out a whole series of activities necessary for the survival of the species: raising offspring, gathering, and processing food, etc. behaving in this way like an organism. Bees are eusocial insects with close interaction with their environment. For this reason, the health of bees is impacted by the effects produced during the collection of nectar and pollen. Nonetheless, a poor nutrition, especially at the end of winter or early spring (lack of microelements, carbohydrates, protein substances, vitamins) and inadequate food sources (pollen soaked with agrochemical and biocides products) of bee colonies, can cause a microbiological dysbiosis; therefore, leading to a decreased ability of the colonies to respond to the environment factors. Bees have a lower diversity of detoxification genes than the genome of other insects. For this reason, to degrade potentially toxic molecules, bees can also rely on other components, which shape their physiology, such as the intestinal microbiome [1,5,9].

It is known that the environment plays a major role in shaping the bee microbiome. Agricultural lands which are being treated with different chemical substances (pesticides, insecticides), contribute to the disruption of the bacterial status in the bees' gut, increasing the vulnerability of bee colonies to different infectious germs (viruses, bacteria, fungal) or to different specific parasites. Therefore, to protect the bee colonies, it is recommended to be placed in less humid or shaded locations the apiaries with more sensitive bee colonies [8, 10]. The composition of the microflora of bee colonies also varies

depending on the place of collection and type of the collected pollen. In pollen, there are various symbiotic communities of microbes that provide a variety of benefits to bees. Microbes associated with pollen supplies are promontory of bee health, but can also represent a major food resource for developing bee larvae. At the same time, the diversity and composition of the intestinal microbiota in bees, differs depending on the ecosystem in which they operate [6,9].

According to scientific data, the composition of the microbiome in bees and bee products consists mainly of lactic bacteria from the genera Lactobacillus and Bifidobacterium, which form a favorable symbiotic environment [2]. The composition of species and the number of bacteria in the intestinal micromyoma of bees depends on several factors: the season, the environment, the source, the quantity and quality of the nectar, the state of the bee, the presence of microorganisms in the nectar [3,10].

The bees and the lactic acid microflora mutually evolved from each other: the bacteria receive a niche with available nutrients, and the bees receive protection from harmful microorganisms. In order to maintain a balanced microbial status and a satisfactory physiological resistance in the bee colonies, as well as to reduce the risk of apathy of some infectious diseases,

it is necessary to systematically monitor the bee colonies. This can be achieved by taking samples for examination of the microbial status, as well as strengthening the physiological status of the bees' body through the additional feeding of biologically active preparations.

In this context, the goal of this proposed research was focused on the use of exometabolites of micromycetes to increase the physiological resistance of bee colonies after the winter period, as well as to stimulate bees productive indices [5,7].

MATERIAL AND METHODS

The researches were carried out in the microbiology laboratory of the Department of Food Safety and Public Health, of the Faculty of Veterinary Medicine of TUM, in the laboratory of the National Collection of the Institute of Microbiology and Biotechnologies. As a material for investigations served the bee families from the experimental apiary of the Institute of Microbiology and Biotechnology, UTM. In orer to obtain the exometabolites from micromycetes from the National Collection of Nonpathogenic Microorganisms, were selected 21 strains of micromycete which were isolated from the soil of the central area of the Republic of Moldova. As nutrient culture mediums for the isolation and the study of morphologycal properties of the strains of micromycetes were used Malt-agar and Czapek mediums. To preserve the micromycetes in the collection of microorganisms, were used the malt-agar medium.

The cultivation of isolated micromycete strains was carried out in a thermostat at a temperature of 28oC for 14 days. The cultures were examined visually according to the morpho-cultural characters, as well as microscopically. The antimicrobial properties of the micromycete isolates were studied according to the diffusometric method by using agar blocks. The method was based on the diffusion capacity of the metabolites produced by the studied microorganisms in the depth of the agar and the action of the active substance in the diffusion zone on the test cultures. The morphological properties of the micromycete strains were studied over 4, 7 and 14 days of cultivation. From the 21 strains of micromycetes used for the investigation of the bee families were selected 3 strains (Ps.sp.11; Ps.sp.19; Ps.sp.62) which demonstated the best development parameters on culture media, as well as more pronounced antibacterial and antifungal actions.

The biomass of exometabolites was prepared from the mentioned strains of micromycetes, and was administered with cakes from wheat flour which were thoroughly homogenized and placed in the hives on the honeycombs to be consumed by the bees.

RESULTS AND DISCUSSION

In order to increase the resistance of the bees' body after the winter period and to stimulate the productive parameters; additional food consisting of cakes of wheat flour with exometabolite of micromycetes biomase were given to bee families. The results of these investigations are presented in table no. 1. where can be seen that were formed 3 experimental groups whith 9 hives in each and one control group. To the bee families from the first group were administered exometabolites of the micromycete strain Ps.sp.11 on 3 dilutions, respectively 10ml, 25ml and 50ml of exometabolites per 1kg of wheat cakes. To the bees from the second group were administered exometabolites of the micromycete strain Ps.sp.19 with delutions 10 ml, 25 ml and 50 ml of exometabolites per 1 kg of wheat cake, and to the families of bees from the third group were administered exometabolites of the micromycete

strain Ps.sp. 62 with delutions of 10 ml, 25 ml and 50 ml of exometabolites per 1 kg of wheat cakes.

At 12, 24 and 36 days after the administration of exometabolites of micromycetes were examined the number of brood squares of the bees in the experimental groups, compared to the bees from the control group.

Analyzing the data presented in table 1, it was determined that the highest index of the number of plots with brood was in the first experimental group of bees, to which was administered the biomass of exometabolites with the strain of micromycetes Ps. sp.11. This index was at 24 days after the administration of the food supplement in a dose of 25ml/kg/cake mass constituted 40.4 squares of brood of bee, compared with 24.7 squares of brood of bee in the control group.

The highest index of - 47.1 squares of brood of bee was established in the first experimental group at 24 days after the administration, representing a diference of 19.4 squares of brood of bee when compared to the control group.

If making a global comparison with the strains of micromycetes Ps. sp. 19 and Ps.sp.62 at 24th day after the administration of exometabolites with indications in the dilution of 25ml/kg/cake mass, this represents an increase of 7.97 and 11.17 squares of brood of bee since they had indices of 35.67 and respectively 38.87 squares of brood of bee.

Table 1

The results of the action of exometabolites of micromycetes to the number of squares of brood of bee

Mycr. strain	Ps. sp.11			Ps.sp.19			Ps.sp.62			Control group		
Dilution / Days	10ml/l	25ml/l	50ml/l	10ml/l	25ml/l	50ml/l	10ml/l	25ml/l	50ml/l	I	II	III
12 days	39,50	40,40	40,25	27,87	28,16	28,33	35,50	36,50	27,60	24,75	28,75	29,00
24 days	41,07	47,10	44,25	35,37	35,67	36,33	34,87	38,87	35,30	27,7	29,25	30,25
36 days	39,93	43,10	42,37	27,62	28,67	31,00	34,50	32,63	28,80	26,25	27,62	27,38

Another indicator that was monitored in the bee families that were additionally fed with the exometabolite biomass was the amount of honey collected from the bee of experimental hives, compared to the control group.

The results of this study are presented in table no. 2. After performing the study, it was determined that the highest collection of honey in the bee families of the first experimental group where the biomass of exometabolites of the strain Ps.sp.11 was administered as additional feed in the dose of 25ml/kg/ wheat cakes was recorded at 24 days after feeding and the collection per beehive constituted 3.4 kg of honey.

Table 2

The amount of honey collected on frames from the beehives of the additional feeding of bee families with the biomase of micromycete exometabolites.

Mycr. Str.	Ps.sp.11			Ps.sp.19			Ps.sp.62			Control group		
Dilu-tion Days	10ml/l%/kg	25ml/l%/kg	50ml/l%/kg	10ml/l%/kg	25ml/l %/kg	50ml/l%/kg	10ml/l%/kg	25ml/l%/kg	50ml/l%/kg	I %/kg	II %/kg	III %/kg
12 days	104 2,6	112 2,8	108 2,7	108 2,7	112 2,8	108 2,7	88 2,2	92 2,3	100 2,5	92 2,3	108 2,7	100 2,5
24 days	109,8 3,0	124,5 3,4	113,5 3,1	106,2 2,9	113,5 3,1	113,5 3,1	98,9 2,7	100 2,5	112 2,8	95,2 2,6	106,2 2,9	98,9 2,7
36 days	121,2 3,6	131,3 3,9	127,9 3,8	104,3 3,1	111,1 3,3	128,2 3,5	109,8 3,0	106,2 2,9	104,3 3,1	97,6 2,9	104,3 3,1	97,6 2,9

In the bee families that were fed with exometabolites biomass from the micromycete strain Ps. sp.19 the honey collection was 3.10kg per beech. At the same time, in the third experimental group of bees which was feed with biomass of exometabolites of strain Ps.sp.62 the honey collection constituted 2.5kg per beehive. In the bees families of the control group, the amount of honey collected from one beehive was 2.6 kg. In conclusion, the difference of amount of honey per beehive in the bee families from the first experimental group was with 0.8kg more per beehive, compared to the bee families from the control group.

Another index that was monitored in the bee colonies after the administration of exometabolites from micromycetes consisted in establishing the degree of prolificacy in dynamic. The results of this study are presented in table 3.

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Table 3

Indices of the prolificacy of the bee families under the action of exometabolites of steptomycetes.

Mycr. strain	Ps. sp.11			Ps.sp.19			Ps.sp.62			Con-trol group
Dilution Days	10ml/l, %	25ml/l, %	50ml/l,%	10ml/l,%	25ml/l,%	50ml/l,%	10ml/l,%	25ml/l,%	50ml/l,%	--, %
12 days	75,67	77,39	77,11	53,40	53,95	54,27	68,01	69,90	52,87	52,68
24 days	78,69	90,23	84,77	67,77	68,33	69,60	66,81	74,47	67,62	55,71
36 days	76,49	82,57	81,18	52,92	54,92	59,38	66,09	62,51	55,17	51,98

The data in the table 3 shows that the highest prolificacy index - 90.23%, was established in the first experimental group of bees, at 24 days after the administration of the biomass of exometabolites of the micromycete with strain Ps.sp.11 in the dilution of 25ml/kg of wheat flour cakes. At the same time, in the second and third experimental groups, to which were administered the biomass of the stains of Streptomyces's Ps. sp.19 and Ps.sp.62, at 24 days after administration, the prolificacy index was 68.33% and 74.47%, respectively. If comparing the experimental groups, the difference in prolificacy between first and second experimental groups was 21.13% and 15.76%, respectively. However, if comparing the first experimental group with the control group, the index of prolificacy was 34.52% higher.

CONCLUSION

1. In order to increase the resistance of the bees' body after the winter period and to stimulate the productive parameters, it is recommended to administer the exometabolites of micromycetes as additional food in the mixture with wheat flour cakes, placed inside the hives on the honeycombs.
2. The use of exometabolites of micromycetes of the strain Ps.sp 11 in a dose of 25ml/kg/ wheat cakes, stimulated the formation of seedlings representing an increase of 24.7 squares of brood of bee if compared with the control group.
3. Feeding the bee families with exometabolites of micromycetes supplements increased the prolificacy index by 21.13%, and the honey collection by 0.8kg per beehive, compared to the bee families from the control group.

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