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THE CONTAMINATION OF BEE POLLEN BY MICROSCOPIC FUNGI

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Annotation. *Culture signs of fungal colonies showed that one of them* develops *on the Sabouraud and Czapek agars, the second - on the Sabouraud and MacConkey agars and the third - on the MacConkey and Czapek agars. In this case, two selected isolates did not grow on the Czapek agar. Despite the fact that calculations of the isolated fungal amount coincide as on the Czapek agar as well on all three agars (200 colony forming units per 1 g of the sample). If we use only Czapek agar,* we *can't identify all spectrum of micromycetes and establish a genus of the fungi, and we can't to evaluate the safety of bee pollen's mycotoxicity.*

Key words: ***bee pollen, dietary supplements, microscopic fungi.***

Bee pollen is widely used as a dietary supplement as in Ukraine so and abroad. This product is normalized by a number of microbiological parameters which determine its sanitary safety - such as the absence of pathogenic staphylococcus and enterobacteria in the samples, the level of contamination of the finished product by mesophilic facultative anaerobic bacteria, microscopic fungi and yeast.

Analysis of the literature shows that the study of bee pollen samples collected in 2002-2004 years did not comply with standards for the number of molds and saprophytic micromycetes in 83-100% samples [1]. In the most pollen samples were recorded exceeding this indicator by 1.4-3.3 times compared with the norm (not more than 100 CFU/g).

The reason for identifying low quality pollen parties may not be as non-compliance technologies of collection, transportation and storage of dietary supplements as natural circumstances - specificity of its epiphytic microbiota composition. Filosfera of plants inhabited a huge number of microorganisms, including microscopic fungi (genera Cladosporium, Penicillium, Altemaria, Aspergillus, Trichoderma, Mucor), used for nutrition plants such as the allocation of carbohydrates and amino acids [2-5]. The formation of the mycobiota of flowering plants affected by several factors: species composition of soil micromycetes and filoplany of plants, geographical area of plant growth, humidity, precipitation, temperature, etc. [6]. Therefore, the study of microbiological contamination of pollen produced in Ukraine, to describe its quality is certainly important issues.

The purpose and objectives of research - to identify the level of contamination by microscopic fungi of various kinds of bee pollen samples that are produced in apiaries of Ukraine.

Materials and methods of research. The material for the study was bee pollen from different regions of Ukraine (center - Cherkasy, Vinnitsa and Poltava region; west - Khmelnytsky and Zakarpattya region; east - Kharkiv and Donetsk region; north - Sumy and Chemihiv region; south - Mykolaiv and Odessa region). Microbiological research of samples was conducted by seeding depth, diluted suspensions of pollen to a number of agar nutrient mediums. To detect microscopic fungi used Czapek and Sabouraud agar. After incubation at 35°C were conducted fungal colonies quantity calculations and analysis of their specific culture characteristics.

Research results. Samples (52 pcs.) were selected during the summer 2013 in different regions of Ukraine: in the center - 19 samples, in the south - 13, in the north - 10, in the east - 4, in the west - 6. By organoleptic characteristics all samples were a solid lumps of irregular shape, yellow, brown or red, with a specific, sweet, pleasant smell that are characteristic features for bee pollen. Mechanical impurities, signs of fermentation or affection by harmful insects were not observed.

Physico-chemical indixes (mass fraction of flavonoid compounds, the concentration of И + ions in 2% solution and the index of oxidability by national standart 3127-95) conform with the standards only in 36.5% (19 samples). The level of microbial contamination was tested in two or three quality samples from each region (Table).

Physico-chemical and microbial contamination characterization of bee pollen samples

Region	Na of the sample	Location in Ukraine	Indexes by national standart			
			pH of 2% solution (4,3-5,3)	amounts of flavonoids (no less than 4,5 %)	oxidability index (no more than 22s)	micro-scopic fungi (100 CFU/g)
Sumy	16	North	5,00	10,2	3	200
Chernihiv	42		5,30	4,5	22	100*
Cherkasy	3	Center	5,21	4,8	5	600
Vinnitsa	12		5,00	4,5	10	1200
Poltava	17		5,10	5,6	10	400
Khmelnytsky	46	West	4,80	11,7	12	80*
Zakarpattya	48		4,50	8,3	18	100*
Mykolaiv	44	South	4,40	5,7	16	333
Odessa	45		4,38	4,7	14	200
Kharkiv	27	East	5,00	6,0	4	100*
Donetsk	52		4,40	8,3	9	400

'Note. Compliance with standards samples concerning contamination by fungi.

In them were not found the characteristic colonies of staphylococcus, coli forms, salmonellas and yeast. Only facultative anaerobic bacteria and micromycets of different species grew on the agar in Petri dishes.

Assessing the level contamination of the samples showed that 63.6% of them do not conform requirements of national standart 3127-95 on the number of microscopic fungi.

We found that agar Czapek is not an optimal medium for colonies of fungi in the calculation of diluted suspensions of bee pollen in conducting the microbiological researches. Using this medium promotes the development of large colonies, making it difficult to identify fungi with a lower rate of reproduction (Figure 1').

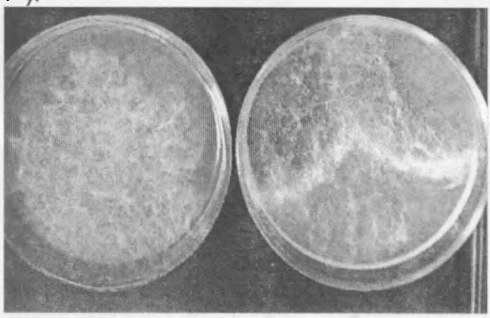

Fig. 1. Growth of colonies on Czapek agar

Suitable for allocation of fungi samples were also Sabouraud agar and MacConkey M081 agar (with CV and 0.15% bile salts), despite the fact that it is a selective medium for isolation of Enterobacteriaceae. On the surface of these substrates grew clear, limited colony that had cultural features slightly different from signs of Czapek agar's colonies (Figure 2).

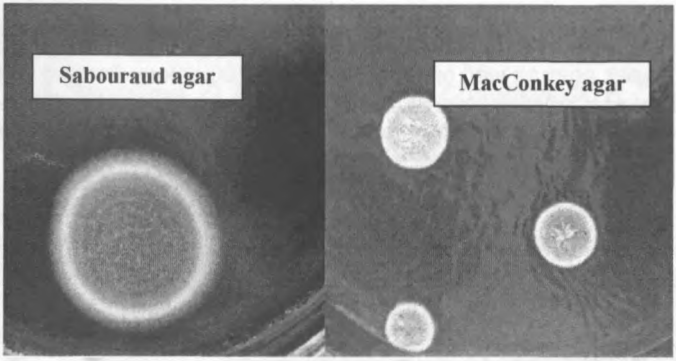

Fig. 2. The growth of colonies on Sabouraud and MacConkey agars

Comparison of cultural signs of fungal colonies grown from one sample showed that the maximum fullness detection of fungal range sample can be while using all these mediums. (Figure 3).

The figure 3 shows that in every agar were developing fungi of two - three species. Studying the cultural signs colonies showed that one of them develops on the Sabouraud and Czapek agars, the second - on the

Sabouraud and MacConkey agars and the third - on the MacConkey and Czapek agars. In this case, two selected isolates did not grow on the Czapek agar.

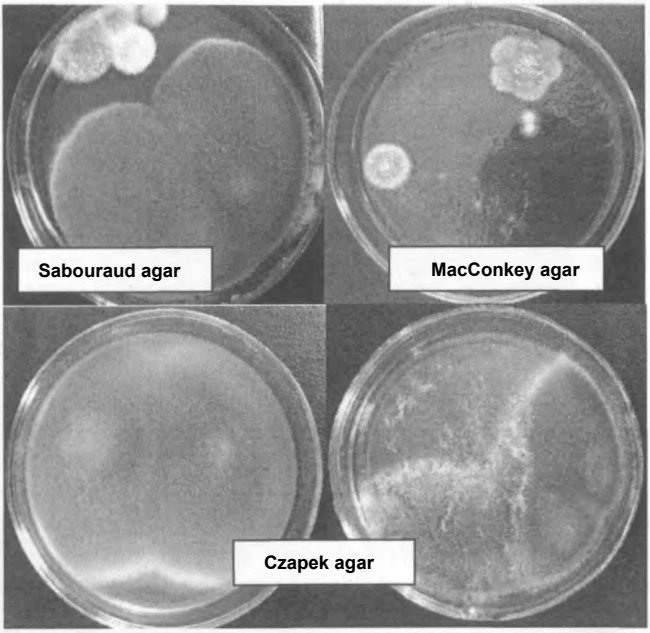

Fig. 3. The fungi growth from bee pollen samples in the case of three types of agar mediums

Despite the fact that calculations of the isolated fungal amount coincide as on the Czapek agar as well on all three agars (200 colony forming units per 1 g of the sample). If we use only Czapek agar, we can't identify all spectrum of micromycetes and establish a genus of the fungi, and we can't to evaluate the safety of bee pollen's mycotoxicity.

Conclusions

It's advisable to make changes in the national standard 3127-95 of bee pollen to use the special media for culturing fungi and to adjustment the fungal contamination rate (100 CFU/g) in the direction of increase.

Prospects for further research. Additional studies will be conducted to establish the real indicator of fungal contamination.

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КОНТАМІНАЦІЯ БДЖОЛИНОГО ОБНІЖЖЯ МІКРОСКОПІЧНИМИ ГРИБАМИ

О. О. *Застулка, О. М. Якубчак, П. О. Солодка, О. Є. Галатюк*

Анотація. ***Вивчення культуральних ознак колоній грибів показало, що одна з них розвивається на агарах Сабуро і Чапека, друга - на Сабуро і Мак Конкі, третя - на середовищах Мак Конкі і Чапека. При цьому, два виділених ізоляти взагалі не росли на агарі Чапека. Незважаючи на те, що обрахунки кількості грибів виділених як на агарі Чапека, так і на всіх трьох середовищах, збігаються (200 КУО на 1 г зразка), використання лише агару Чапека не дозволяє виявити спектр мікроміцетів і встановити родову належність грибів, а також оцінити безпеку бджолиного обніжжя щодо мікотоксичності.***

Ключові слова: *бджолине обніжжя, БАД, мікроскопічні гриби.*

КОНТАМИНАЦИЯ ПЧЕЛИНОЙ ОБНОЖКИ МИКРОСКОПИЧЕСКИМИ ГРИБАМИ

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Аннотация. ***Изучение культуральных признаков колоний грибов показало, что один из них развивается на агар Сабуро и Чапека, вторая - на Сабуро и Мак Конки, третья - на средах Мак Конки и Чапека. При этом, два выделенных изолята вообще не росли на агаре Чапека. Несмотря на то, что расчеты количества грибов выделенных как на агаре Чапека, так и на всех трех средах совпадают (200 КОЕ на 1 г образца), использование только агара Чапека не позволяет выявить спектр микромицетов и установить родовую принадлежность грибов, а также оценить безопасность пчелиной обножки по микотоксичности.***

Ключевые слова: *пчелиная обножка, БАД, микроскопические грибы.*