



ANTIBACTERIAL ACTIVITY OF BEE POLLEN



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Introduction

Bee pollen, as well as other apicultural products, has gained increased attention for its therapeutic properties, as antibacterial (Garcia et al., 2001) antifungal (Garcia et al., 2001), anticarcinogenic (Almas et al., 2001) and immunomodulatory (Gebera et al., 2002) effects. Bee gathered pollen is considered a valuable special food with varied enhancing effects in health (Bogdanov, 2004). Antibacterial activity of pollen is studied by many authors (Hass, 1992; Campos et al., 1998; Bassim et al., 2005; Carpes et al., 2007). Bee pollen is the bee product least influenced by contaminants from beekeeping. It is important to control the microbiological quality of pollen, especially relation of fungi and mycotoxins following the legislation applied for food (Gonzales et al., Campos et al. 2008).

The purpose of the present studies is determination of the antibacterial activity of bee pollen loads basically from two kinds of flower (*Brassica napus* and *Heliantus anus*) and level of contamination by filamentous fungi

Materials and Methods

Samples for antibacterial analysis

Antibacterial activity of 41 samples of bee pollen water extracts obtain basically from two kinds flower (*Brassica napus* and *Heliantus anus*) was study against following species of bacteria : MRSA - *Staphylococcus aureus* ST2013, *Enterobacter aeruginosa* SH2001, *Salmonella enteridis* SA-312, *Bacillus cereus* B-34, *Klebsiella pneumoniae* K-12, some strains form genus *Lactobacillus* (*L. Rarnosus* L-56) and "pink" yeasts (*Rhodotorula minuta* W-24). Mentioned starins are isolated from different foodstuffs. Average samples of ethanol extracts of bee pollen (PEE) were also studied from the mentioned plants. In analysis have been tested different concentrations of PEE.

Determination of antibacterial activity

Stock cultures of bacteria were grown in Nutrient Broth (HiMedia) at 26-27°C for 24h in a shaker. Final cell concentrations were 10⁷-10⁸ cfu/ml. The paper disc diffusion method was used to detect the antibacterial activity of pollen water and ethanol extracts. Sterilized filter paper discs were soaked with 20 µl of pollen ethanol extracts (PEE). The absolute ethanol used as a negative control. For determination antibacterial activity of water pollen extracts was used well diffusion method. The plates were incubated at 27°C and observed after 48h. At the end of the period, inhibition zones around the paper disc were measured. All tests were done in triplicate.

Samples pollen for mycological analysis

A total of 45 bee pollen samples were studied. Thirty samples of them were obtained from Slovakian producers. The remaining ten samples from Armenian producers and five from Russian producers.

Determination of water activity

Determination of a_w was spent with AquaLab (Decagon Devices, Pullman, WA, USA). Definition pH was spent with pH-meter (Oakon, USA).

Isolation and identification of fungi

Five gram of sample was suspended in 50 ml of sterile water. From the first suspension series of decimal dilutions were performed and used to inoculate Petri dishes containing GYA, Chapek - DOX Agar (Himedia, India). Dishes were incubated during 8 days at 25-30°C in the dark. After this time, the contamination level of samples (CFU/g of sample) by the different fungi was evaluated. All assays were performed in triplicate. Taxonomic identification of all isolates was achieved through macroscopic and microscopic observation with the aid of guidelines published for each genus (Barnett and Hunter, 1972; Klich and Pitt, 1988, 1991).

Conclusion

- Antibiotic activity of bee pollen in most times is defined by a kind of plants;

- Different samples of bee pollen collected at various time from the same plant possessed different activity;

- Antagonistic activity of bee pollen can depend on such factors as climatic and geographical conditions in which the plant grows, time of gathering of pollen, technologies of drying which is necessary to consider at manufacturing of preparation from bee pollen ;

- According to the results obtained, pollen seems to have interesting biological properties, and can be considered as a functional food;

- However, more studies are needed to create a well based ground for setting maximum limits for CFU/g as regards toxicogenic fungi.



Results

Antibacterial activity of pollen water extract (PWE) analyzed was according to the well diffusion assay. The PWE of *Brassica napus* pollen at 50% shows some inhibition degree against MRSA - *St. aureus* ST2013 and *E. aeruginosa* SH2001. None of water extracts of bee pollen samples have shown antagonistic activity against lactic acid bacteria, *Rhodotorula minuta*, *Bacillus cereus*, *Klebsiella pneumoniae* and *Salmonella enteridis* SA-312.

The sensitivity of the bacteria decreased in the order for *Heliantus anus* : *Enterobacter aeruginosa* > *St. aureus* > *Salmonella*, for *Brassica napus* :

E. aeruginosa > *E. coli* > *St. aureus* > *Bacillus cereus*.

Pollen grains have specific characteristics according to the flora species or cultivation methods, but the quality depends on the collection process, cleanness, drying and storage applied by bee keepers with the objective to increase the product shelf-life. The quality of bee pollen is strongly depend on its preservation.

There were spent mycological analysis of 35 samples of bee pollen, received from the sunflower and repts in basic. 26 species and 11 genus of micromycetes , which divided in two classes - *Zygomycetes* and *Deuteromycetes* are allocated and identified.



Inhibition of *St. aureus* by pollen *Heliantus anus*



Control of antibiotics against *St. aureus*



Inhibition of *E. aeruginosa* by pollen, *Senecio*, *Phacelia*, *Papaver*, *Trifolium*, *Senecio*

It is shown dependence of level of contamination of bee pollen by fungi from water activity. The samples of bee pollen are more subjected to contamination by fungi potential producers of mycotoxins. The composition of species of micromycetes , isolated from *Brassica napus* is more various then from *Heliantus anus*.

Water activity (aw) of bee pollen that ready for human consumption is about 0.268 (0.261-0.280) (Serra, 1997). The lower aw requirements of *Aspergillus* spp. and *Penicillium* spp. would explain their higher occurrence frequency in bee pollen. It is well known that some species of these two genera, isolated from other substrates, are considered to be the most significant toxicogenic moulds.

Fungi-potential producers of mycotoxins , isolation from bee pollen

Species of fungi	Mycotoxins	Bee pollen from <i>Brassica napus</i>	Bee pollen from <i>Heliantus</i>
<i>A. flavus</i>	Aflatoxins	208	
<i>A. nomius</i>	Aflatoxins		205
<i>A. tubigenensis</i>	Ochratoxin A		206
<i>A. carbonarius</i>	Ochratoxin A		204
<i>A. ochraceus</i>	Ochratoxin A	208	
<i>A. nidulans</i>	Sterigmatocystin	203	
<i>A. fumigatus</i>	Tremorgenic	201, 203, 211	
<i>P. fellutanum</i>	Patulin	201, 211, 210	

Antibacterial activity of bee pollen water extracts (PWE) (50%)

Bee pollen from	Number of samples	Antibacterial activity (in mm) against					
		<i>St. aureus</i> ST2013			<i>E. aeruginosa</i> SH2001		
		Min	Max	Average	Min	Max	Average
<i>Brassica napus</i>	11	5.0	16.0	11.45	4.5	20.0	7.1
<i>Heliantus anus</i>	10	6.5	30.0	14.1	5.0	20.0	8.0
<i>Brassica napus</i>	1	-	-	10.0*	-	-	0
<i>Heliantus anus</i>	1	0	6.5	6.5*	-	5.0	5.0
Papaver	2	14	20.0	17.0	0	23.0	11.5
Trifolium	2	0	17.0	8.5	10.0	33.0	17.5
Phacelia	3	0	13.0	4.3	8.0	20.0	14.0
Senecio	2	0	6.0	3.0	5.0	24.0	17.5
Senecio	2	10.0	20.0	15.0	0	0	0
Armenian (polliflor)	5	0	10.0	4.4	0	0	0
Russian (tankovna)	3	0	15.0	7.0	0	0	0
Control A (gentamicin)	-	-	-	16.0	-	-	18.0

Antibacterial activity of *Brassica napus* and *Heliantus anus* pollen ethanol extracts (PEE) (inhibition zone diameter in mm)

Pollen extract (PEE)	<i>Bacillus cereus</i>	<i>St. aureus</i>	<i>E. aeruginosa</i>	<i>E. coli</i>
H1/5	8	9	12	9
H1/10	6	7	10	7
H1/25	0	6	8	0
H1/50	0	0	6	0
H1/100	0	0	0	0
H1/5	11	12	9	7
H1/10	10	11	7	6
H1/25	9	10	6	0
H1/50	7	7	0	0
H1/100	0	0	0	0
Control A (oxytetracyclin)	13	18	15	16

Fungi contaminating of bee pollen

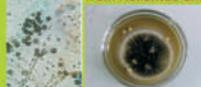
Species of fungi	Bee pollen from <i>Brassica napus</i>	Bee pollen from <i>Heliantus anus</i>
<i>Mucor circinelloides</i>		204, 205, 206 (12.5%)
<i>M. caryocarpus</i>	207	206 (8.3%)
<i>M. mansueti</i>		205 (4.1%)
<i>M. lamprosporus</i>	208	
<i>Mortierella</i> spp.	207	
<i>Trichoderma</i>		
<i>Mortierella subelliptica</i>	208	
<i>Rhizopus nigricans</i>	203, 202, 209	212 (16.6%)
<i>Aspergillus flavus</i>	208	
<i>A. nomius</i>		205
<i>A. carbonarius</i>	204	
<i>A. fumigatus</i>	201, 203, 211	
<i>A. nidulans</i>	203	
<i>A. multicolor</i>	210	
<i>A. niger</i>	207	206
<i>A. bisporus</i>	202	
<i>A. sclerotiorum</i>	204	
<i>A. foetidus</i>		
<i>Penicillium aurantio-erythrum</i>	203, 211	
<i>P. fellutanum</i>	201, 210	
<i>P. pharis</i>	202	
<i>Scopulariopsis chrysomphala</i>	201	
<i>Ferriellium subellipticum</i>	207	
<i>Parasartaria olivacea</i>	201	
<i>Alternaria alternata</i>	201	212
<i>Ustilidium</i>	210	
<i>Mycelia sterilia</i>	201, 210	

Interrelation between level of contamination by filamentous fungi and water activity

Samples of bee pollen	aw	Level of contamination by fungi, cfu/g
<i>Heliantus anus</i>	0.579-0.638	3x10 ⁵ -2x10 ⁶
<i>Brassica napus</i>	0.505-0.644	10 ⁴ -7x10 ⁵
Senecio, Salsdago, Phacelia	0.414-0.490	3x10 ³ -5x10 ⁴

The received results have shown about existence of a problem of pollution bee pollen by fungi among which kinds are marked species of producers mycotoxins .

A. Carbonarius growth on pollen from *Heliantus anus*



A. flavus growth on *Brassica napus*



The samples of bee pollen from *Heliantus anus* with high level of water activity

